NOCTURNAL ASTHMA IN CHILDREN

W.M.C. van Aalderen

NOCTURNAL ASTHMA IN CHILDREN

W.M.C. van Aalderen

STELLINGEN

1. Verslechtering van de 08.00 uurs PEFR-waarde bij kinderen met CARA is een indicatie voor toename van de nachtelijke bronchusobstructie.

2. Een PC_{10} histamine is even reproduceerbaar als een PC_{20} histamine bij de bepaling van de mate van bronchiale hyperreactiviteit bij allergische kinderen met CARA.

3. De ernst van nachtelijke bronchusobstructie wordt door kinderen met CARA en hun ouders onderschat.

4. Toegenomen nachtelijke bronchusobstructie bij allergische kinderen met CARA wordt, in tegenstelling tot bij volwassenen met CARA, niet veroorzaakt door toename van de parasympatische activiteit.

5. De adrenerge respons na inspanning bij allergische kinderen met CARA is omgekeerd evenredig met de ernst van de ziekte.

6. Het staken van onderhoudsmedicatie bij allergische kinderen met CARA kan aanleiding geven tot verslechtering van de longfunctie 's nachts, waardoor de klinische verslechtering pas laat herkend wordt.

7. Een "happy wheezer" kan moeilijk "gewoon happy wezen".

8. Ook bij jonge kinderen met CARA zijn beta-receptoren aanwezig en functioneel.

9. Zieke kinderen die tachycard, tachypnoeisch en perifeer slecht gecirculeerd zijn, zijn in shock tenzij het tegendeel bewezen is.

10. Het inzichtelijk maken van het rookgedrag van nederlandse huisartsen heeft binnen enkele jaren geleid tot een halvering van het aantal rokers binnen die groep.

11. Gezien de toename van operatieve mogelijkheden valt het te verwachten dat er bij kinderen van moeders met een gecompliceerde congenitale hartafwijking, een groter herhalingsrisico van gecompliceerde congenitale hartafwijkingen bestaat dan nu bekend is.

12. Bronchiolitis bij oudere zuigelingen is een uiting van CARA.

Groningen, 22 maart 1989 W.M.C.van Aalderen.

RIJKSUNIVERSITEIT GRONINGEN

Nocturnal asthma in children

Proefschrift

Ter verkrijging van het doctoraat in de Geneeskunde

aan de Rijksuniversiteit Groningen
op gezag van de Rector Magnificus Dr. L.J. Engels
in het openbaar te verdedigen op woensdag 22 maart 1989

des namiddags om 2.45 uur precies

door

Willem Marinus Christiaan van Aalderen

geboren 15 februari 1952

te Den Haag.

Promotores:	Prof.Dr. K. Knol
	Prof.Dr. G.H. Koëter
Referent :	Dr. D.S. Postma

Promotiecommissie:	Prof.Dr. H.S.A. Heymans
	Prof.Dr. H.J. Sluiter
	Prof.Dr. K. de Vries

The studies in this thesis were supported by a grant (no.85.36) from the "Nederlands Astma Fonds".

Fisons Pharmaceuticals (Leusden, the Netherlands) did the excellent job to make this thesis broadly available to all who are interested.

To everything there is a season and a time to every purpose under the heaven: a time to be born and a time to die; a time to plant, and time to pluck up that which is planted

Ecclesiastes 3 1 and 2

Zetwerk en layout: Letter & Lijn, Groningen.
Drukwerk: Krips Repro, Meppel.

CONTENTS

VOORWOORD

Dit onderzoek werd verricht binnen de afdeling Kinderlongziekten van de Kinderkliniek van het Academisch Ziekenhuis te Groningen. Tevens werd intensief samengewerkt met de afdelingen Longziekten en Allergologie van de Kliniek voor Inwendige geneeskunde te Groningen.

In de eerste plaats wil ik de kinderen bedanken, die spontaan en ten alle tijden enthousiast aan het onderzoek hebben meegewerkt. Tevens wil ik hun ouders bedanken voor het gestelde vertrouwen.

Prof.Dr. K. Knol gaf mij de stimulans die uiteindelijk tot dit proefschrift leidde. Beste Klaas, jij hebt mij enthousiast gemaakt voor zowel het klinische vak Kinderlongziekten als voor onderzoek op dit gebied. Ik wil je bedanken voor de vriendschappelijke sfeer waarin overleg ten alle tijden en over vele onderwerpen kon plaats vinden.

Veel dank ben ik verschuldigd aan Prof.Dr. G.H. Koëter voor de opbouwende en kritische discussie bij het opstarten en bij het verdere verloop van dit onderzoek. Beste Gerard, ik heb veel geleerd van je onderzoekskwaliteiten. Ik dank je voor de hulp die je mij als vanzelfsprekend gaf en die dit onderzoek tot een vlot einde gebracht heeft.

Dr. D.S. Postma ben ik zeer erkentelijk voor de voortdurende steun en begeleiding bij het tot stand komen van dit proefschrift. Beste Dirkje, je stuwende kracht, en je kennis op het gebied van nachtelijke kortademigheid bij CARA patienten, was van grote waarde voor dit proefschrift.

Prof.Dr. K. de Vries ben ik erkentelijk voor zijn steun aan dit project. Beste Klaas, bedankt dat je een luisterend oor hebt gegeven op momenten waarop de toekomst van dit onderzoek erg somber scheen. Je grote kennis op het gebied van CARA en van 24 uurs ritmes waren van grote waarde.

Prof.Dr. H.J. Sluiter wil ik bedanken voor zijn belangstelling voor dit onderzoek en voor het stimuleren van de goede samenwerking tussen de afdelingen Longziekten en Kinderlongziekten.

Drs. L.Th.v.d.Weele ben ik zeer erkentelijk voor de hulp en uitleg bij de statistische bewerking van de meetgegevens. Leo, bedankt dat je de statistiek wat begrijpelijker voor mij maakte.

Dr. Jorrit Gerritsen wil ik bedanken voor het kritisch evalueren van een deel van dit proefschrift. Beste Jorrit, bedankt voor de prettige samenwerking, en de bereidheid om de kliniek op noodzakelijke momenten over te nemen.

Dr. Jan G.R.de Monchy en Dr. Peter J.Sterk wil ik bedanken voor het kritisch evalueren van delen van dit proefschrift.

Prof.Dr. H.S.A. Heymans, beste Hugo, je hebt de voorwaarden en het klimaat gegarandeerd waarin dit proefschrift tot een vlot einde gebracht kon worden, mijn dank hiervoor.

Het Nederlands Astma Fonds wil ik bedanken voor het mogelijk maken van mijn opleiding in de kinderlongziekten. De Wetenschappelijke Advies Raad van het Nederlands Astma Fonds wil ik bedanken voor het kritisch evalueren van het studie-protocol en voor de goede en nuttige adviezen die hieruit voortvloeiden.

Aaf Teuben wil ik bedanken voor het vele werk ook 's nachts, aan dit project. Aaf, mede door jouw is dit onderzoek in een uitstekende sfeer verlopen.

Nienke Leever wil ik bedanken voor haar hulp bij het typen en de secretariele ondersteuning van dit project.

Henk Breukelman van het Centraal Klinisch Chemisch Laboratorium van het Academisch Ziekenhuis Groningen en Ep Oosting van het Bergschot Centre for Research in Breda wil ik bedanken voor de vlotte bepaling van respectievelijk de catecholamines en het N^{τ}-methylhistamine in de vele urine porties.

Rob Thunnissen, Hennie Knoester en Ruurd Jan Roorda wil ik bedanken voor het overnemen van de klinische werzaamheden ten tijde van het schrijven van dit proefschrift.

De afdeling kindercardiologie wil ik danken voor het mogen gebruiken van de electrocardiograaf.

De heer F.G. Sluiter wil ik bedanken voor het corrigeren van mijn gebruik van de engelse taal.

Gerrit Messchendorp en Lodewijk Martijn dank ik voor de perfecte tekeningen die zij maakten om dit proefschrift te illustreren.

Greet van Rijn en Titie Dijkstra van de afdeling diëtetiek wil ik bedanken voor het bepalen van alle diëten.

De medewerkers van het A-laboratorium van de kinderkliniek wil ik bedanken voor de gastvrijheid en de hulp bij het verwerken van de urine monsters.

Jan van der Steen van de afdeling kinderpsychiatrie wil ik bedanken voor de plezierige manier waarop hij de introductie- gesprekken met de gezonde kinderen liet verlopen.

Zuster Holwerda wil ik bedanken voor het feit dat de kinderen die aan dit onderzoek meededen, ook steeds in de kliniek ondergebracht konden worden. Ida, bedankt.

Het personeel van de afdeling grote kinderen wil ik bedanken voor hun hulp bij het volbrengen van dit onderzoek, en de gastvrije sfeer waarin de kinderen ontvangen en begeleid werden.

Prof.Dr. J. Fernandes en Prof.Dr. C.A. Winkel wil ik bedanken voor mijn opleiding in de kindergeneeskunde.

Mijn ouders ben ik dankbaar voor de gelegenheid die ze mij boden om te studeren. Samen met mijn schoonouders zijn zij steeds een steun voor ons.

Tenslotte dank ik Ina, Michiel en Mart Jan voor hun geduld en voor de tijd die ze me gegeven hebben om dit onderzoek te verrichten en dit boekje te schrijven. En inderdaad Mart Jan: pappa woont thuis.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Chronobiology

Claude Bernard described the concept of homeostasis of an organism in 1865.[1] This French investigator indicated that the internal milieu of an organism is kept constant by several regulatory mechanisms which maintain a steady state. Around this mean state, however, the functions of an organism fluctuate. In fact, these fluctuations represent natural and probably genetically determined rhythms. These rhythms have been documented on all levels of physiological organization from unicellular organisms up to mankind.

In man, several different rhythms can be observed and the period of these rhythms may differ widely, for instance, from cycles of fractions of seconds in the electroencephalogram up to cycles of about one year in duodenal ulcers.

The study of alterations of each organism's temporal structure in various situations is called chronobiology.[2]

Biological rhythms mainly consist of cycles of about 24 hours (circadian) and of about one year (circannual). Also other bioperiodicities, e.g. of less than 20 hours (ultradian), approximately 7 days (circaseptan), 20 days (circavigintan), or 30 days (circatrigintan) are recognized. Periods of more than 28 hours are called infradian.

Although cyclic changes in organisms have been known for centuries, it was the French astronomer J.J. de Mairan who reported in 1729 that the circadian changes of the heliotrope, a flower, persisted in constant darkness.[3]

Cyclic variations only partly reflect the endogenously determined rhythm. External factors, the so-called synchronizers, time cues, or "Zeitgebers", adjust the endogenous rhythms in such a way, that they remain in phase with their surroundings. For clear-cut identification of an endogenous rhythm, this rhythm must be studied under constant environmental conditions, and after removal of any known synchronizer. Under these conditions the period of the rhythm usually deviates from that of the synchronized environmental cycle. This phenomenon is called free-running.[4]

It is important to realize that synchronizers are only capable of changing the expression of the rhythm, they do not create rhythms.

Rhythms are present at birth, and are stable during adulthood. The amplitude of a rhythm (i.e. the difference between the highest and lowest value of a cycle expressed as percentage of the highest value) may decrease with age as has been shown for potassium excretion and growth hormone levels. Some rhythms even disappear. Other rhythms continue throughout life, for instance the rhythm in plasma cortisol.[5]

After initial observations of circadian variations in plants, wide-ranging research in the field of chronobiology started.

1.2. Circadian rhythm in pulmonary function in asthmatic patients

A circadian variation in pulmonary function, with peak and trough values at 16.00 and 04.00 hours respectively, exists in diurnally active normal subjects, and in asthmatic patients (patients with sudden attacks of diffuse, but reversible airflow obstruction).[6,7] The activity-sleep schedule is one of the main synchronizers of circadian rhythm in man. The diurnal rhythm in peak expiratory flow rate (PEFR) values in normal people has a mean amplitude of 8%.[7-9] A similar circadian rhythm is observed in asthmatic patients, but with a greately enhanced amplitude. The mean PEFR is generally below predicted. This is usually associated with a reduction in both peak and trough values, but is sometimes produced solely by a fall in overnight peak flow rates. This shows that nocturnal asthma represents an exaggeration of the normal rhythm in airway diameter.[7,10]

Nocturnal and early morning dyspnea are common symptoms in children and adults with asthma.[11-13] Episodes of dyspnea occur about 40 times more frequently overnight than during the day.[14] The nightly increase in airflow obstruction is observed in one third of the patients with asthma.[13] It is an annoying symptom, which causes the patients to wake up at night, and in children may lead to poorer performance at school. Nocturnal dyspnea may prove difficult to treat.[13,15] In adult asthma, the nocturnal increase in airflow obstruction is found to be a risk factor for death.[16-18]

An increased nocturnal airflow obstruction has already been described in the fourth century AD.[19] Since then, many reports have been written on the subject. In the 17th century Willis attributed it to the bedclothes overheating the blood, necessitating a "more plentiful sucking in of air".[19] In 1698 Floyer found that his attacks were exclusively nocturnal for over seven years and in 1827 Laënnec described a patient whose attacks only occurred if his night-light went out.[19] In 1868 Trousseau described nocturnal asthma in both himself and his mother.[19] In the fourties and fifties of the present century, research into determinants of nocturnal asthma began, to which the Groningen group contributed.

Although nocturnal dyspnea in asthmatics has been recognized for ages, it is only recently that some insight has been obtained in endogenous factors underlying the nocturnal fall in pulmonary function, such as circadian variation in bronchial hyperresponsiveness, the autonomic nervous system, and exogenous factors such as exposition to allergens, gastroesophageal reflux, mucociliary clearance, and body position.

1.3. Factors contributing to an increase in airflow obstruction overnight

1.3.1. The autonomic nervous system

The airway diameter is under control of the autonomic nervous system consisting of the parasympathetic nervous system, the orthosympathetic nervous system, and the non-adrenergic, non-cholinergic nervous system (NANC).

The parasympathetic system

Resting airway calibre is influenced by vagal tone.[20] Stimulation of irritant receptors in the walls of the trachea and the large airways excite the efferent fibres of the parasympathetic bronchoconstricting nervous system. The efferent fibres run in the vagal nerve up to the nicotinic receptor in the parasympathetic ganglia in the wall of the bronchial tree. From this ganglion, post-ganglionic fibres lead to bronchial smooth muscle and mucosal glands.[21] The neurotransmitter of this muscarinic receptor is acetylcholine. The bronchoconstricting effect of acetylcholine can be blocked by atropine or atropine-like substances, e.g. ipratropium bromide.

Vagal stimulation causes bronchial constriction and hypersecretion of the mucus glands. Stimulation of muscarinic receptors, also present on mast cells, increases release of mediators from these cells and thereby enhances permeability of the airways leading to edema, inflammation, and airflow obstruction.

Not only the airways, but also the human heart is under vagal control. The heart shows a circadian rhythm, with its lowest frequency between 02.00 and 06.00 hours at night.[22] Direct measurements of parasympathetic activity in humans is not possible for ethical reasons. Changes in heart rate (HR) and a parameter that is more closely related to vagal tone, sinus arrhythmia gap (SAG), reflect the vagal activity.[22,23] These parameters are therefore frequently used to measure parasympathetic activity.

In non-allergic patients with chronic obstructive lung disease, Postma et al.[9] observed an overall increase in SAG in comparison with their matched healthy controls. This same phenomenon was found in asthmatic adults.[24] These studies describe an increase in SAG overnight in the patient groups, suggesting a nocturnal increase in vagal activity that could be responsible for the increased airflow obstruction overnight.

Although recent investigations suggest that anticholinergic drugs may prevent nocturnal dyspnea in adults with asthma,[25,26] the results of other studies, especially in children, do not agree with this observation.[27-29] It is thus still questionable whether the parasympathtic nervous system in asthmatic children is involved in increased airflow obstruction overnight.

The orthosympathetic system

In contrast to the parasympathetic nervous system, sympathetic innervation of the airways is scanty, with no direct adrenergic supply to airway smooth muscle.[30-32] Adrenergic nerves may, however, modulate bronchomotor tone indirectly, via an effect on cholinergic nerves and ganglia. Alpha- and beta-adrenergic receptors are in this respect the most important parts of the orthosympathetic system. In normal subjects, both systems contribute little to the regulation of the airway diameter.

The alpha-adrenergic system

$Alpha_1$-receptors are present in the normal human lung.[33] The role of this group of receptors in regulating the diameter of the bronchi in asthmatic patients is controversial.[34,35] Although asthmatics exhibit an exaggerated bronchoconstrictor response to inhaled agonists, the issue of circadian differences has not been specifically addressed.

The beta-adrenergic system

The beta-adrenergic system can be regarded as a counter- regulatory system that may lead to bronchodilatation in asthmatic patients.[36-39]

The beta-adrenergic system consists of circulating catecholamines, and the beta-receptor adenylate cyclase complex. Adrenaline is secreted by the adrenal medulla, noradrenaline is primarily released from axon terminals of sympathetic postganglionic neurons, and appears in the plasma mainly as a result of overflow of sympathetic nerve activity. These hormones, as well as $ß_2$-adrenergic drugs, play an important role in stimulating the beta-receptor adenylate cyclase complex. After binding to the receptor on the outer surface of the plasma membrane, the adenylate cyclase on the inner surface is activated, thereby stimulating the conversion of ATP to c-AMP, thus starting a cycle of reactions ultimately leading to relaxation of the bronchial smooth muscle.

Catecholamines

Even though the resting plasma adrenaline level is not elevated in asthmatics,[40] the fact that bronchoconstriction develops with beta-blockers suggests a protective role of adrenaline in airflow obstruction.

A circadian rhythm in urinary catecholamines has been observed in normal subjects,[41,42] with lowest values found in the period from 23.00 to 07.00 hours. A comparable observation was made in asthmatic patients by Soutar et al.[43], and Barnes et al.[40], showing a circadian variation in circulating catecholamines. In the latter study, both patients with asthma and their controls showed similar adrenaline values at all

points in time. The asthma group showed an increase in circulating histamine at the nadir of the circulating adrenaline level. This negative correlation between histamine and adrenaline did not exist in the control group. Moreover, after adrenaline infusion in the asthmatic group in low concentrations at 04.00 hours, compensating for the nocturnal decline in adrenaline, plasma histamine concentrations fell, coinciding with a rise in PEFR values. This suggests that a reduced inhibition of histamine release from sensitized mast cells by adrenaline might underlie the nocturnal fall in pulmonary function in asthma.

In a group of non-allergic patients with chronic obstructive lung disease, and decreasing FEV_1 values overnight, Postma et al.[44] observed a decline in nocturnal adrenaline and noradrenaline urinary excretions. This decline was also found in the control group. Both adrenaline and noradrenaline excretion were, however, significantly reduced compared with the controls, indicating that in non-allergic older patients impaired adrenaline secretion may lead to increased airflow obstruction. But the increased airflow obstruction overnight does not appears to be the result of a fall in catecholamines overnight.

Beta-adrenergic receptor function

Since Szentivanyi postulated that a generalized beta-adrenergic dysfunction underlies the pathogenesis of asthma,[45] different studies have shown that reduced metabolic and cardiovascular reponses to catecholamines may exist in asthma.[46-48] Furthermore, reduced beta-adrenergic receptor numbers or function have been observed in leukocytes and lung tissue.[49-53] Nevertheless, this reduced beta-adrenergic receptor function is probably not intrinsic to the disease, but may be elicited by external factors such as viral respiratory tract infections, allergen exposure, or frequent use of beta-adrenergic drugs.[54-60]

Studies concerning the role of the beta-receptor function in nocturnal asthma show conflicting results.

Titinchi et al.,[61] showed a circadian variation in lymphocyte beta-adrenergic receptor density of asthmatic patients, with a relatively low density at 08.00 hours and a relatively high density at 18.00 hours. This circadian variation was also observed in a normal control group.

In lymphocytes of non-allergic patients with chronic airflow obstruction, Meurs et al.[62] observed no significant diurnal variation in beta-adrenergic receptor number (measured at 08.00 hours during the fall in lung function in the morning, and at 16.00 hours when lung function is maximal), neither in the patients group, nor in the controls. A similar result was obtained for the isoprenaline-induced cAMP production. As these patients and normals were somewhat older (mean age 53 years) as studies in asthmatic patients, this suggests that the circadian rhythm of beta-adrenergic response alters with age.

Although differences in circadian variation in receptor density are observed in different patient populations, any decrease in receptor density does not seem not to be of major importance to nocturnal asthma since Barnes et al.[63] showed that the airway, cardiovascular, and plasma cAMP responses after incremental infusions of adrenaline, measured every 4 hours over 24 hours, were similar at all times of observation, indicating that there is no evidence that responses of the airways to infused or inhaled adrenaline are impaired during the night.

The non-adrenergic, non-cholinergic nervous system

Next to the autonomic parasympathetic and sympathetic nervous systems controlling the level of airway tone in asthmatic patients, the non-adrenergic non-cholinergic nervous (NANC) system, which is present in animals and humans, contributes to the bronchial smooth muscle tone.[64]

This system consists of components with an inhibitory or excitatory influence on human airway smooth muscle. NANC fibres probably constitute the only direct inhibitory innervation of human airway smooth muscle.[65-67] As in parasympathetic nerves, NANC nerve fibres run via the vagal nerve and synapse of ganglia in the walls of the larger airways. The neurotransmitters of this inhibitory system are probably vasoactive intestinal peptide and the peptide histidine isoleucine.[68-70]

Excitatory nerves of this system have been identified in various animal species as well as in humans.[71] NANC-airflow obstruction is due to release of neuropeptides (e.g. substance P and other tachykinins, neurokinins, neuropeptide K, and Calcitonine gene-related peptide) from C-fibre endings.[72] Barnes proposed that when these sensory endings were more exposed to epithelial-cell-damaging inflammatory mediators, an axon reflex might be triggered, resulting in smooth muscle contraction, microvascular leakage, and mucus hypersecretion.[73]

Untill now, no studies have been performed to investigate the relation between increased airflow obstruction overnight and the NANC nervous system.

1.3.2. Bronchial hyperresponsiveness

Bronchial hyperresponsiveness is defined as the abnormal sensitivity of the airways to physical, chemical, and pharmacological stimuli,[74] and is a characteristic feature of asthmatic patients.[75,76] De Vries et al. [77] and Tammeling et al.[78] were among the first to demonstrate an increased bronchial hyperresponsiveness on histamine challenge during the night at 20.00, 24.00 and 04.00 hours in "asthmatic" and "bronchitic" patients with nocturnal dyspnea. Forced expiratory values in one second (FEV_1) were lowest at 24.00 and 04.00 hours. Investigations by Reinberg et al.[79], using acetylcholine, confirmed this circadian rhythm in bronchial hyperresponsiveness in asthmatic patients.

As both bronchial hyperresponsiveness and airflow obstruction increase at night, the question arises whether an increase in bronchial hyperresponsiveness at night may be the result of increased airflow obstruction. In non-allergic "emphysematous" patients, Sluiter et al.[80] showed an increase in bronchial hyperresponsiveness during the night, while nocturnal FEV_1 values remained at day-time levels, suggesting that in these patients bronchial hyperresponsiveness was independent of the level of airflow obstruction. Another explanation for this observation, however, might be that the airflow obstruction in these patients is already severe and, more importantly, irreversible. Moreover, findings may be different in allergic asthmatic patients.

The precise mechanism underlying hyperresponsiveness of asthma is unknown. The possibility that airways inflammation could be related to the development and maintenance of the bronchial hyperresponsiveness in asthma has been the subject of increasing research in recent years. Inflammation of the airways may create conditions that have themselves been proposed as possible mechanisms of hyperresponsiveness, such as bronchial edema, mucosal hyperpermeability, exposure of epithelial sensory nerve endings, and the release of mediators, such as histamine.[74]

Both histamine and serotonine, amine-derivates of histidine and tryptamine, have for a long time been known as bronchoconstrictors. Histamine is known to be a constituent of mast cell granules and of other mediator cells. This mediator may be released in the lung, or it may act on airway smooth muscle following systemic degranulation. The mast cell seems also to play an important roll in bronchial hyperresponsiveness, as the level of histamine released from these cells in vitro is correlated with the degree of airway hyperresponsiveness, as assessed by PC_{20} histamine.[81] Moreover, the pulmonary mast cell in situ response to inhaled allergens is greater in allergic asthmatics than in normals, as measured by histamine and tryptase levels in the broncho-alveolar lavage fluid.[82] Baseline or spontaneous mast cell activation appears also to be greater in allergic asthmatics. In contrast to histamine, serotonin is only a mild bronchoconstrictor, and like histamine leads to vasodilatation.

1.3.3. Serum cortisol levels

Corticosteroids are well-known for their beneficial effects in restoring pulmonary function in severe asthma attacks. The circadian variation in serum cortisol levels has long been recognized. Although decreasing cortisol levels during the night precede the nocturnal fall in FEV_1 values in asthmatic patients, and a relationship is assumed between them, Soutar et al.[83] showed that cortisol infusion did not abolish the nocturnal fall in pulmonary function. Moreover, Postma et al.[9] observed comparable cortisol levels in non-allergic patients with chronic obstructive lung disease (patients with persistent, but variable airflow obstruction) with an increase in nocturnal airflow obstruction, and their matched normal controls. These observations suggest that the

nocturnal fall in endogenous cortisol can not be regarded as the primary cause of nocturnal asthma.

1.4. Exogenous factors contributing to increased airflow obstruction overnight

1.4.1. Allergen exposure

Immunoglobuline E is the major immunoglobulin of allergic diseases, including allergic asthma. Large circadian variations in total immunoglobulin E have been found in allergic asthmatic patients.[84] Plasma IgE is highest at midday and lowest during the night. The day-night difference is presumably indicative of a temporal difference in tissue-bound IgE, where low plasma IgE levels at night presumably coincide with elevated tissue-bound IgE, indicating that mast cell degranulation, and inflammatory reactions in the lung, may increase overnight.

It is generally accepted that intensive contact with mites in the home and in beddings may lead to early and late obstructive reactions (EOR, LOR respectively).[85,86] Furthermore, inhalation of allergens may result in increased nocturnal airflow obstruction for many nights.[87,88] On the other hand, reduction of allergen concentration has been shown to decrease, but not abolish, bronchial hyperresponsiveness and early morning dyspnea.[89] This indicates in allergic asthmatic patients that exposure to allergens is important for the development of nocturnal airflow obstruction. In asthmatic patients, Gervais et al.[90] observed that the fall in FEV_1 values after a similar dose of inhaled allergens overnight was greater than during the day, indicating that an increased responsiveness of the airways overnight to allergens in this group of patients may also contribute to nocturnal asthma.

In non-allergic patients with chronic airflow obstruction, nocturnal dyspnea may also be an annoying symptom.[9] This shows that exposure to allergens is certainly not the only factor that determines the nocturnal fall in pulmonary function.

1.4.2. Sleep

Studies in asthmatic subjects working in a shift system showed that the rhythm in PEFR values adjust after shift change. The PEFR values are lowest on waking, irrespective of the actual time of day. The individual time of adjustment may vary considerably.[91] Five asthmatic patients doing shift work showed rapid adjustment after shift change, so that the rhythm in PEFR had fully adjusted to the change in shift by the time the first normal sleep had taken place after the shift change. This might suggest that sleep itself is responsible for the fall in PEFR values noted on waking. Subsequent studies in a further 9 patients showed, however, that some individuals adapt more slowly.

These findings and many clinical observations suggest that nocturnal dyspnea is

related to sleep. On the other hand, airflow obstruction in asthmatics with nocturnal dyspnea is not directly reversed after waking up, and may persist for some time afterwards. This indicates that nocturnal asthma is not directly related to sleep itself, since parameters such as breathing pattern and ventilatory control return to waking level directly after awakening.[92-95]

Studies attempting to relate the phase of sleep and the periods of wheezing are inconclusive. In asthmatic adults, Kales et al.[95] observed that attacks were randomly distributed throughout the various phases of sleep. In asthmatic children, fewer attacks during electroencephalogram (EEG) stages III and IV were noted.[96] As no objective lung function measurements were performed in this study, there is a possibility that these children slept through their attacks.

In 11 asthmatic subjects, Hetzel et al.[91] compared PEFR values after intermittent awakening with PEFR values when these subjects were kept awake until after the time at which the nocturnal fall in PEFR values would have been expected (03.00 - 05.00 hours). They then slept until 06.00 hours. In six subjects the nocturnal fall in PEFR equalled the fall that had been observed previously. In the other five, however, no fall in PEFR values was observed until after awakening at 06.00 hours. The results of this study are contradictory.

The same study protocol was also used by Catterall et al.[97], but in this study, sleep was confirmed by EEG measurements. Airflow obstruction occurred on both nights, but the mean nocturnal fall in PEFR values was greater on the "asleep" night than the "awake" night, suggesting that sleep itself may have some influence on nocturnal asthma.

The association between sleep and clinical variation in asthma is likely to be the result of two basic circadian rhythms with similar phase relationships and entrained by common synchronizers such as rest, arousal or the light-dark cycle.

1.4.3. Gastroesophageal reflux

Gastroesophageal reflux (GER) has been reported to occur in a high percentage of children with asthma. The reported incidence of reflux in these children varies from 47% to 63%.[98,99] The mechanism that is responsible for this high incidence in childhood is not yet clear, but several possible mechanisms have been suggested.[100-102]

Recurrent wheeze was found in 18% of a group of 126 children with GER.[103] The relationship between GER and respiratory symptoms was suggested on the basis of an improvement in symptoms following anti-reflux therapy.[104-107] In a group of asthmatic children with nocturnal dyspnea, Martin et al.[108] observed that a higher reflux score corresponded to a higher number of nights during which the children wheezed. This correlation was only minimal in a group of patients who mainly had complaints of wheezing in daytime. Unfortunately, no pulmonary function measurements were perfor-

med to establish circadian rhythmicity in both groups.

The aspiration of gastric contents, or reflux of acid into the esophagus at night, when acid secretion in the stomach is particularly great,[109] causes nocturnal bronchoconstriction. Other studies confirm that the passage of irritating solutions through the esophagus may influence the degree of airflow obstruction and bronchial hyperresponsiveness. In a double blind study by Wilson et al.[110], a group of asthmatic children was challenged with a drink of diluted hydrochloric acid (0.001 N); a significant increase in mean histamine hyperresponsiveness 90 minutes after ingestion of the fluid was observed. No change in baseline PEFR could be observed. The pattern of increased bronchial hyperresponsiveness without significant change in baseline PEFR values was also very similar to that reported after a cola drink,[111] ingestion of tartrazine capsules,[112] as well as ice.[113] Other investigations showed that a more powerful stimulus of hydrochloric acid (0.1 N) instilled into the esophagus in the presence of oesophagitis can induce an immediate increase in respiratory resistance in asthmatic subjects.[114]

A vagal reflex seems to be the most likely explanation for the occurrence or increase of airflow obstruction after GER or irritating substances, since in dogs with induced gastroesophagitis an increase in pulmonary resistance can be abolished after section of the vagal nerve.[115]

As acid or gastric reflux also occurs in healthy subjects, it can only be regarded as a trigger factor in nocturnal airflow obstruction.

1.4.4. Mucociliary Clearance

In normal as well as in asthmatic subjects, Bateman et al.[116,117] observed a reduced mucociliary clearence during the night. Posture did not affect mucociliary clearance, as clearance in erect and supine position was similar. As mucus production is increased in asthmatic patients, it is possible that retention of mucus also contributes to nocturnal dyspnea in asthmatic patients. It is certainly not a major factor, since inhaled beta-$_2$ adrenergic drugs quickly reverse nocturnal bronchoconstriction.

1.6. Aims of the studies

Underlying mechanisms of nocturnal dyspnea, and their importance, are unknown or controversial. Most of the studies on the subject compare asthmatic patients with nocturnal dyspnea, with normal controls. Nocturnal dyspnea is, however, not a separate disease, as the term "nocturnal asthma" suggests, but one of the symptoms asthmatic patients may suffer from. It seems, therefore, relevant to investigate aspects of the subject in a group of asthmatic patients with, and in a group of asthmatic patients without nocturnal increase in airflow obstruction.

Therefore we selected two groups of patients with asthma. Except for the difference in amplitude of pulmonary function parameters, we tried to make the two groups as homogeneous as possible with regard to age and pulmonary function level. The results of investigations in these two groups of asthmatic children were compared with an age-matched group of normal controls.

In the first part of this study we investigated whether the amplitude of the circadian PEFR values after three days of withdrawal of maintenance treatment could be predicted using parameters such as daytime FEV_1 and PEFR values during medication, and/or the drugs needed to control the symptoms of the patients satisfactorily. As pulmonary complaints and daytime PEFR measurements are easely measured signal functions for deterioration of the disease state in the home situation, we investigated whether these parameters could predict the occurence of a nocturnal dip in pulmonary function. Moreover, we were interested whether the first signs of deterioration in the disease state were pulmonary complaints, or a decrease of PEFR values.

An autonomic disbalance has been frequently considered to be the underlying mechanism of nocturnal airflow obstruction. Therefore we investigated circadian changes in vagal activity and urinary catecholamine excretion in all three groups. Moreover, urinary N^{τ}-methylhistamine excretion (an indicator for histamine release) was measured to determine whether inflammatory mediators could contribute to nocturnal airflow obstruction.

Many investigators have found that the challenge dose of histamine which causes a 20% fall in FEV_1 from baseline, correlates in asthmatic patients with the prechallenge FEV_1 or FEV_1/FVC ratio, especially when FEV1 is below 70% of the predicted value. An increase in bronchial hyperresponsiveness during the night, at the very moment that the FEV_1 is lowest, may thus be simply the consequence and not the cause of the nocturnal fall in pulmonary function. Therefore we investigated the relationship between the level of pulmonary function and the degree of bronchial hyperresponsiveness during 24 hours.

The last few years several reports in asthmatic individuals have described both an early and late bronchial obstructive reaction (EOR and LOR, respectively) upon exercise challenge. [118,119] This reaction pattern had already been known for a long time after allergen challenge.[86] We hypothesized that patients with nocturnal airflow obstruction more frequently had LORs to antigen or exercise exposure than patients without nocturnal airflow obstruction. We investigated whether differences between the two groups of asthmatic children existed in airflow obstructive patterns after house dust mite inhalation and after exercise. We also investigated whether these possible differences

between the two groups were caused by differences in catecholamine response, or by differences in N^{τ}-methylhistamine excretion.

In addition to investigations concerning nocturnal dyspnea ,we tried, with the same patient group, and without changing the study design, to answer a question that has been asked for a long time in our department.

In the past, bronchial hyperresponsiveness was expressed as a provocation concentration (PC) of histamine causing a 10% and 15% fall in baseline FEV_1 values (PC_{10} and PC_{15}, respectively). More recently, for several reasons, a PC_{20} histamine has been used in the assessment of bronchial hyperresponsiveness. To compare past and present methods, we investigated whether a PC_{10} and PC_{15} are as reproducible as a PC_{20} histamine in the assessment of the degree of bronchial hyperresponsiveness in asthmatic patients.

REFERENCES

1. Bernard C. Introduction à l'étude de la médicine expérimentale. JB Ballière et Fils, Paris 1865.
2. Halberg F, Carandente F, Cornelissen G, Katinas GS. Glossery of chronobiology. Chronobiologia 1977;4 suppl. 1:189-98.
3. Reinberg A, Smolensky MH. Introduction to chronobiology. In: Reinberg A, Smolensky MH, eds. Biological Rhythms and Medicine. Cellular, Metabolic, Physiopathologic and Pharmacologic Aspects. New York, Inc. Springer-Verlag 1983; 1.
4. Halberg F, Siffre M. Engeli M, Hilmann D, Reinberg A. Etude en librecours des rhythms circadien du pouls, de l'alternance veillesommeil, et de l'estimation du temps pendant les deux mois de sejour souterrain d'un homme adulte jeune. Comptes Rendus Acad.Sci. 1965;260:1259-62.
5. Rietveld WJ. Clinical Aspects of Chronobiology: Introduction. In: Rietveld WJ ed. Clinical Aspects of Chronobiology. Baarn. 1984;5.
6. Gaultier C, Reinberg A, Girard F. Circadian rhythms in lung resistance and dynamic lung compliance of healthy children. Effects of two bronchodilators. Respiration Physiology 1977;31:169-82.
7. Hetzel MR, Clark TJH. Comparison of normal and asthmatic circadian rhythms in Peak expiratory flow rate. Thorax 1980;35:732-8.
8. Kerr HD. Diurnal variation of respiratory function independent of air gravity. Arch Environ Health 1973;26:144-52.
9. Postma DS, Keyzer JJ, Koëter GH, Sluiter HJ, De Vries K. Influence of Parasympathetic and Sympathetic nervous system on nocturnal bronchial obstruction. Clin Sci 1985;69:251-8.
10. Reinberg A, Gervais P. Circadian rhythms in respiratory functions with special reference to human chronophysiology and chronopharmacology. Bull Physiopath Res 1972;8:663-72.
11. Sly PD, Hibbert ME, Landau LI. Diurnal Variation of Peak Expiratory Flow Rate in Asthmatic children. Pediatr Pulmonol 1986;2:141-6.
12. Clark TJH, Hetzel MR. Diurnal variation of asthma. Br J Dis chest 1977;71:87-92.
13. Connolly CK. Diurnal Rhythms in Airway obstruction. Br J Dis Chest 1979; 73:357-66.
14. Dethlefsen U, Repges R. Ein neues Therapie-prinzyp bei nächtlichen Asthma. Med Klin 1985;80:44-47.
15. Turner-Warnick M. On observing patterns of airflow obstruction in chronic asthma. Br J Dis Chest 1977;71:73-86.
16. Cochrane GM, Clark TJH. A survey of asthma mortality in patients between ages 35 and 64 in the greater London hopitals in 1971. Thorax 1975;30:300-5.

17. Hetzel MR, Clark TJH, Branthwaite MA. Asthma: analysis of sudden deaths and ventilatory arrests in hospital. Br Med J 1977;I:808-11.
18. Anonymous. Asthma at night. (Editorial) Lancet 1983; I:220-2.
19. Hetzel MR. The pulmonary clock. Thorax 1981;36:481-6.
20. Nadel JA, Barnes PJ. Autonomic regulation of the airways. Annual Review of Medicine 1984;35:451-67.
21. Al-Walid I, El-Barmani AW, Grant M. Acetylcholinesterase -positive nerves of rhesus monkey bronchial tree. Thorax 1975;30:162-70.
22. Clarke JM, Hamer J, Shelton JR, Taylor S, Venning GR. The rhythm of the normal human heart. Lancet 1976;II:508-12.
23. Carruthers M, Taggart P. Vagotonicity of violence: biochemical and cardiac responses to violent films and television programmes. Br Med J 1973;3:384-9.
24. Kallenbach JM, Webster T, Dowdeswell R, Reinach SG, Scott Millar RN, Zwi S. Heart rate control in asthma. Evidence of parasympathetic overactivity. Chest 1985;87:644-8.
25. Morrison JFJ, Pearson SB, Dean HG. Parasympathetic nervous system in nocturnal asthma. Br Med J 1988;296:1427-9.
26. Cox ID Hughes DTD, McDonnell KA. Ipratropium bromide in patients with nocturnal asthma. Postgr Med J 1984;60:526-8.
27. Catteral JR, Rhind GB, Douglas NJ. Nocturnal asthma is not caused by increased airway vagal tone. (Abstract) Am Rev Resp Dis 1985;131(suppl):55a.
28. Sly PD, Landau LI, Olinski A. Failure of ipratropium bromide to modify the diurnal variation of asthma in asthmatic children. Thorax 1987;42:357-60.
29. Gaultier C, Reinberg A, Girard F. Circadian rhythms in lung resistance and dynamic compliance of healthy children. Effects of two bronchodilators. Respiration Physiology 1977;31:169-82.
30. Richardson JB, Ferguson CC. Neuromuscular structure and function in the airways. Fed Proc 1979; 38: 202-8.
31. Davis C, Kannan S, Jones TR, Daniel EC. Control of human airway smooth muscle: in vitro studies. J Appl Physiol 1982; 53: 1080-7.
32. Kirkpatrick CT. Nervous control of airway muscle. Bull Eur Physiolpathol Respir 1984; 20: 389-94.
33. Barnes PJ, Karliner JS, Dollery CT. Human lung adrenoreceptors studied by radioligand binding. Clin Sci 1980;58:457-61.
34. Barnes PJ, Ind PW, Dollery CT. Inhaled prazosin in asthma. Thorax 1981;36:378-81.
35. Thomson NC, Daniel EE, Hargreave FE. Role of smooth muscle alpha$_1$-receptors in non-specific bronchial responsiveness in asthma. Am Rev Respir Dis 1982; 126:521-5.
36. De Vries K, Gökemeyer JDM, Koëter GH, Monchey de JGR, Bork van LE,

Kauffman HF, Meurs H. Cholinergic and adrenergic mechanisms in bronchial hyperreactivity. In: Morley J, ed. Perspectives in asthma I, Bronchial hyperreactivity, Academic Press, London, 1982a:107.
37. De Vries K, Koëter GH, Gökemeyer JDM. Some aspects of the regulation of the bronchial tree in obstructive lung disease. Eur J Respir Dis 1982 ; 63(suppl. 121):60-3.
38. Koëter GH, Meurs H, Kauffman HF, De Vries K. The role of the adrenergic system in allergy and bronchial hyperreactivity. Eur J Resp Dis 1982;63(suppl.121):72-8.
39. Zaid G, Beall GN. Bronchial response to beta-adrenergic blockade. N Engl J Med 1966;275:580-4.
40. Barnes PJ, Fitzgerald G, Brown M, Dollery C. Nocturnal asthma and changes in circulating epinephrine, histamine and cortisol. N Engl J Med 1980;303:263-7.
41. Reinberg A, Halberg F, Ghata J, Gervais P, Abulker C, Dupont J, Gandau C. Rhythme circadien de diverses fonctions physiologiques de l'homme adulte sain, actif et au repos (pouls, pression artérielle, excrétions urinaires des 17-OHCS, des catecholamines et du potassium). J Physiol 1969;61(suppl.2):383.
42. Townsend MM, Smith AJ. Factors influencing the urinary excretion of free catecholamines in man. Clin Sci 1973;44:253-65.
43. Soutar CA, Carruthers M, Pickering CAC. Nocturnal asthma and urinary adrenaline and noradrenaline excretion. Thorax 1977;32:677-83.
44. Postma DS, Koëter GH, Keyzer JJ, Meurs H. Influence of slow release terbutaline on the circadian variation of catecholamines, histamine and lungfunction in non-allergic patients with partly reversible airflow obstruction. J Allergy Clin Immunol 1986;77:471-477.
45. Szentivanyi A. The beta-adrenergic theory of atopic abnormality in bronchial asthma. J Allergy 1968;42:203-32.
46. Inoue S. Effects of epinephrine on asthmatic children. J Allergy 1967;40:337-48.
47. Lockey SD, Glennon JA, Reed CE. Comparison of some metabolic responses in normal and asthmatic subjects to epinephrine and glucagon. J Allergy 1967;40:349-54.
48. Shelhamer JH, Metcalfe DD, Smith LJ, Karliner MJ. Abnormal beta-adrenergic responsiveness in allergic subjects: analysis of isoproterenol-induced cardiovascular and plasma cyclic adenosine monophosphate responses. J Allergy Clin Immunol 1980;66:52-60.
49. Brooks SM, McGowan K, Bernstein IL, Altenan P, Peagler J. Relationship between number of beta-adrenergic receptors in lymfocytes and disease severity in asthma. J Allergy Clin Immunol 1979;63:401-6.
50. Logsdan PJ, Middleton E, Coffey RG. Stimulation of leucocyte adenyl cyclase by hydrocortison and isoproterenol in asthmatic and non-asthmatic subjects. J Allergy Clin Immunol 1972:50:45-56.

51. Parker CW, Smith JW. Alterations in Cyclic AMP metabolism in human bronchial asthma. I: Leukocyte responsiveness to beta-adrenergic agents. J Clin Invest 1973;52:48-59.
52. Paterson JW, Lulich KM, Goldie RG. The role of beta-adrenoceptors in bronchial hyperreactivity In: Morky A, ed. Perspectives in asthma I. Bronchial hyperreactivity, London: Academic Press, 1982;19.
53. Szentivanyi A. The conformational flexibility of adrenoceptors and the constitutional basis of atopy. Triangle 1979;18:109-15.
54. Busse W. Decreased granulocyte response to isoproterenol in asthma during upper respiratory tract infections. Am Rev Respir Dis 1977;115:783-90.
55. Meurs H, Kauffman HF, Timmermans A, Monchy de JGR, Koëter GH, De Vries K. Specific immunological modulation of lymfocyte adenylate cyclase in asthmatic patients after allergenic bronchial provocation. Int Arch Allergy Appl Immunol 1986;81:224-9.
56. Meurs H, Koëter GH, De Vries K, Kauffman HF. The beta-adrenergic system and allergic bronchial asthma: changes in lymphocyte beta-adrenergic receptor number and adenylate cyclase activity after an allergen-induced asthmatic attack. J Allergy Clin Immunol 1982;70:272-80.
57. Meurs H, Koëter GH, De Vries K, Kauffman HF. Dynamics of the lymfocyte beta-adrenoceptor system in patients with allergic bronchial asthma. Eur J Respir Dis 1984;65(suppl.135):47-61.
58. Conolly, Greenacre JK. The lymphocyte beta-adrenoceptor in normal subjects and patients with bronchial asthma. J Clin Invest 1976;58:1307-16.
59. Koëter GH, Meurs H, Kauffman HF, Monchy de JGR, Sluiter HJ. De Vries K. Changes in the beta-adrenergic system in bronchial asthma induced by terbutaline. Agents and Actions. 1983;13(suppl.):259-64.
60. Weber RW, Smith JA, Nelson HS. Aerosolized terbutaline in asthmatics: developement of subsensitivity with long-term administration. J Allergy Clin Immunol 1982;70:417-22.
61. Titinchi S, Al Shamma M, Patel KR, Kerr JW, Clark B. Circadian variation in number and affinity of beta-2 adrenoceptor in lymphocytes of asthmatic patients. Clin Sci 1984;66:323-8.
62. Meurs H, Postma DS, Koëter GH, Keyzer JJ, Kauffman HF, De Vries K. The beta-adrenergic system and circadian bronchial obstruction. In: Kerrebijn KF, Sluiter HJ, eds. Nocturnal dyspnoea, inflammation and reactivity. The Netherlands, Rijswijk: Astra Pharmaceutica BV. 1986;49.
63. Barnes PJ, Fitzgerald GA, Dollery CT. Circadian variation in adrenergic responses in asthmatic subjects. Clin Sci 1982;62:349-54.
64. Barnes PJ. Neural control in human airways in health and disease. Am Rev Resp Dis 1984;134:1289-314.

65. Richardson J, Béland J. Non-adrenergic inhibitory nervous system in human airways J Appl Physiol 1976;41:764-71.
66. Doidge JM, Satchell DG. Adrenergic and non-adrenergic inhibitory nerves in mammalian airways. J Auton Nerv System 1982;5:83-99.
67. Satchell D. Non-adrenergic, non-cholinergic nerves in mammalian airways: their function and the role of purines. Comp Biochem Physiol 1982;72:189-96.
68. Dey RD, Shannon WA, Said SI. Localisation of VIP-immunoreactive nerves in airways and pulmonary vessels in dogs, cats and human subjects. Cell Tissue Res 1981;22:231-8.
69. Palmer JBD, Cuss FMC, Barnes PJ. VIP and PHM and their role in non-adrenergic inhibitory responses in isolated human airways. J Appl Physiol 1986;61:1322-8.
70. Lundberg JM, Saria A. Polypeptide-containing neurons in airway smooth muscle. Ann Rev Physiol 1987;49:557-72.
71. Barnes PJ. Neural control of human airways in health and disease. State of art. Am Rev Resp Dis 1986;134;1289-314.
72. Lundberg JM, Saria A, Lundblad. Bioactive peptides in capssaicin-sensitive C-fibre afferents of the airways: functional and pathophysiological implications. In: Kaliner MA, Barnes PJ, eds. The airways: Neural control in health and disease. New York: Marcel Dekker 1988;417.
73. Barnes PJ. Asthma as an axon reflex. Lancet 1986;i:242-4.
74. Boushey HA, Holtzman MJ, Sheller JR, Nadel JA. Bronchial Hyperreactivity. Am Rev Res Dis 1980;121:389-411.
75. Curry JJ. The action of histamine on the respiratory tract in normal and asthmatic subjects. J Clin Invest 1946;25:785-91.
76. Knol K. A clinical and epidemiological study of the significance of bronchial hyperreactivity in children with chronic non specific lung disease. the Netherlands, Groningen: University of Groningen 1965, 194pp Dissertation.
77. De Vries K, Goei JT, Booy-Noord H, Orie NGM. Changes during 24 hours in the lungfunction and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. Int Arch Allergy 1962;20:93-101.
78. Tammeling GJ, De Vries K, Kruyt EW. The circadian pattern of the bronchial reactivity to histamine in healthy subjects and in patients with obstructive lung disease. In: McGovern JD, Smolensky MH, Reinberg A, eds. Chronobiology in allergy and Immunology. Springfield Ill, Thomas CC. 1977;139.
79. Reinberg A, Gervais P, Morin M, Abulker C. Rhythme circadien humain du seuil de la response bronchique a l'acetylcholine. Comp-Rend, Acad Sci (Paris) 1971;272 :1879-81.
80. Sluiter HJ, Orie NGM, Vegter JJM, Tammeling GJ. Emphysema and Cor Pulmonale. In: Orie NMG, Sluiter HJ (eds). Bronchitis I. Assen: Royal van Gorkum 1961; 233.

81. Flint KC, Leung KBP, Hudspith BN, Brostoff J, Pearce FL, Johnson NM. Bronchoalveolar mast cells in extrinsic asthma: a mechanism for the initiation of antigen specific bronchoconstriction. Br Med J 1985;291:923-6.
82. Wenzel SE, Fowler AA, Schwartz LB. Activation of pulmonary mast cells by bronchalveolar allergen challenge. Am Rev Resp Dis 1988;137:1002-8.
83. Soutar CA, Costello J, Ijaduola O, Turner-Warwick M. Nocturnal and Morning asthma. Relationship to plasma corticosteroids and response to cortisol infusion. Thorax 1975;30:436-40.
84. Gaultier C, Monis de G, Reinberg A, Motohashi Y. Circadian rhythm in serum total immunoglobulin E (IgE) in asthmatic children. Biomed and Pharmacotherapy 1987;41:186-8.
85. Tovey ER, Chapman M, Platts-Mills TAE. Mite faeces are a major source of house dust allergens. Nature 1981; 289:592-3.
86. Van Lookeren-Campagne JG, Knol K, De Vries K. House dust provocation in Children. Scand J Respr Dis 1969; 50:76-85. 87. Newman-Taylor AJ, Davies RJ, Hendrick OJ, Pepys J. Recurrent nocturnal asthmatic reaction to bronchial provocation tests. Clin Allergy 1979;9:213-9.
88. Davies RJ, Green M, Schofield N. Recurrent nocturnal asthma after exposure to grain dust. Am Rev Resp Dis 1976;114:1011-9.
89. Platts-Mills TAE, Mitchell EB, Nock P, Tovey ER, Moszoro H, Wilkins SR. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. Lancet 1982;II:675-7.
90. Gervais P, Reinberg A, Gervais C, Smolensky M. Twenty-four hour rhythm in the bronchial hyperreactivity to house dust in asthmatics. J Allergy Clin Immunol 1977;59:207-213.
91. Hetzel MR, Clark TJH. Does sleep cause nocturnal asthma? Thorax 1979;34:749-54.
92. Douglas NJ, White PD, Weil JV, Pickett CK, Zwillich CW. Hypercapnic ventilatory response in sleep in adults. Am Rev Resp Dis 1982; 126:758-62.
93. Douglas NJ, white PD, weil JV, Pickett CK, Martin RJ, Hudgel DW, Zwillich CW. Hypoxic ventilatory response decreases during sleep in normal man. Am Rev Resp Dis 1982;126:286-9.
94. Douglas NJ, White DP, Pickett CK, Weil JV, Zwillich CW. Respiration during sleep in normal man. Thorax 1982;37:840-4.
95. Kales A, Beall GN, Bajor GF, Jacobson A, Kales JD. Sleep studies in asthmatic adults: Relationship of attacks to sleep stage and time of night. J Allergy 1968; 41:164-73.
96. Kales A, Kales JD, Sly RM, Scharf MB, Tan TL, Preston T. Sleep patterns in asthmatic children: All-night electroencephalographic studies. J Allergy 1970; 46: 300-8.

97. Catterall JR. Rhind GB, Steward IC, Whyte KF, Shapiro CM, ouglas NJ. Sleep deprivation on overnight bronchoconstriction in nocturnal asthma. Thorax 1986;41:676-80.
98. Euler AR, Byrne WJ, Ament ME, Fonkalsrud EW, Strobel CT, Siegel SC, Katz RM, Rachelefsky GS. Recurrent pulmonary disease in children: a complication of gastroesophageal reflux. Pediatrics 1979;63:47-51.
99. Shapiro GG, Christie DL. Gastroesophageal reflux in steroid dependent asthmatic youths. Pediatrics 1979;63:207-12.
100. Goyal RJ, Rattan S. Mechanism of lower esophageal sphincter relaxation action of prostaglandin E, and theophylline. J Clin Invest 1973;52:337-41.
101. Berguist WE, Rachelfsky GS, Kadden M, Siegel SC, Katz RM, Mickey MR, Ament ME. Effect of theophylline on gastroesophageal reflux in normal adults. J Allerg Clin Immunol 1981;67:407-11.
102. Stalcup SA, Mellins RB. Mechanical forces producing pulmonary edema in acute asthma. New Engl J Med 1977;297:592-6.
103. Sheperd RW, Wren J, Evans S, Lander T, Ong H. Gastroesophageal reflux in children. Clinical profile, course and outcome with active therapy in 126 cases. Clin Pediatr 1987;26:55-60.
104. Overolt RH, Ashraf MM. Esophageal reflux as a trigger for asthma. NY State J Med 1966;66:3030-2.
105. Berguist WE, Rachelefsky GS, Kadden M, Siegel SC, Katz RM, Fonkalsrud EW, Ament ME. Gastroesophageal reflux-associated recurrent pneumonia and chronic asthma in children. Pediatrics 1981;68:29-35.
106. Johnson DG, Herbst JJ, Oliveros MA, Stewart DR. Evaluation of gastroesophageal reflux surgery in children. Pediatrics 1977;59:62-8.
107. Goodall RJR, Earis JE, Cooper DH, Bernstein A, Temple JG. Relationship between asthma and gastro-esophageale reflux. Thorax 1981;36:116-21.
108. Martin ME, Grunstein MM, Larsen GL. The relationship of gastroesophageal reflux to nocturnal wheezing in children with astma. Annuals of allergy 1982;49: 318-22.
109. Moore JG, Englert T. Circadian rhythm of gastric acid secretions in men. Nature 1970;226:1261-2.
108. Wilson NM, Charette L, Thomson AH, Silverman M. Gastro-oesophageal reflux and childhood asthma: the acid test. Thorax 1985;40:592-7.
109. Wilson N, Vickers H, Taylor G, Silverman M. Objective test for food sensitivity in asthmatic children: increased bronchial responsiveness after cola drinks. Br Med J 1982;284:1226-8.
110. Hariparsad D, Wilson N, Dixon C, Silverman M. Oral tartrazine in childhood asthma: effect on bronchial reactivity. Clin Allergy 1984;14:81-5.
111. Wilson NM, Dixon C, Silverman M. Increased bronchial responsiveness caused by

ingestion of ice. Eur J Respir Dis 1985;66:25-30.
112. Spaulding HS, Mansfield LE, Stein MR, Sellner JC, Gremillion DE. Further investigation of the association between gastro-esophageal reflux and bronchoconstriction. J Allergy Clin Immunol 1982;69:516-21.
113. Mansfield LE, Hameister HH, Spaulding HS, Smith NJ, Glab N. The role of the vagus nerve in airway narrowing caused by intra-oesophageal hydrochloric acid provocation and esophageal distension. Clin Allergy 1981;47:431-4.
114. Bateman JRM, Pavia D, Clarke SW. The retention of lung secretions during the night in normal subjects. Clinical Science and Molecular Medicine 1978;55:523-7.
115. Bateman JRM, Clarke SW, Pavia D, Sheahan NF. Reduction in clearance of secreations from the human lung during sleep. J Physiol 1978;284:55.
116. Lee TH, Nagakura T, Papageorgiou N, Likura Y, Kay AB. Exercise-induced late asthmatic reactions with neutrophil chemotactic activity. N Engl J Med 1983;308: 1502-5.
117. Bierman CW, Spiro SG, Petherham I. Characterization of the late response in exercise-induced asthma. J Allergy Clin Immunol 1984;74:701-6.

CHAPTER 2

METHODS

Three groups of children were selected to participate in the studies that took place in the hospital. Two groups of allergic asthmatic children were selected from our pediatric outpatient clinic. The third group, a group of normal controls matched for age, was recruited from the children of friends and colleagues.

The study design and the selection of the patients and their controls as described in this chapter refers to the chapters 5, 6, and 7.

Approach to the children

The children were asked to participate in the study in the presence of their parents. At the moment of recruitment the complete study design was explained. Moreover, each separate part of each new procedure that was going to be used on the study day was explained in advance. An observer appointed by the Medical Ethics Committee was present during this first visit.

One of the investigators was always available for advice. The children could withdraw from the study at any desired moment.

Informed consent from all children and their parents was obtained. The study protocol was approved by the Medical Ethics Committee of the University Hospital of Groningen.

Patient selection

Selection criteria were:

1. An amplitude in PEFR values at home measured on three consecutive days of ≥ 20% (Group I) or < 15% (Group II). Amplitude is defined as the difference between the highest and lowest PEFR value measured over 24 hours, expressed as percentage of the best value.
2. A history of episodic wheezing on exposure to allergens and non-allergic stimuli.
3. A forced expiratory volume in one second as percentage of the predicted value (FEV_1 % pred.) ≥ 70%.
4. Increased Total IgE, and at least a positive skin test on house dust mite (HDM) extract (Diephuis Laboratories, the Netherlands).
5. Increased bronchial hyperresponsiveness, defined as a histamine provocation concentration < 16 mg/ml causing a fall of 20% or more in FEV_1 from baseline value (see histamine inhalation provocation).

Asthma symptoms were well-controlled by maintenance medication. The children did not suffer from respiratory tract infections at the time of the study or during the three

months before the study. No oral corticosteroids were used for at least six months before the study.

Selection of the normal controls

Nine healthy children, matched for age to the children of group I, took part in the study. These controls did not use medication, nor was there a previous history of respiratory complaints. All healthy children had normal spirometry, normal blood eosinophil counts, and negative skin tests to house dust mite extract. None of the children showed bronchial hyperresponsiveness (PC_{20} histamine > 16 mg/ml).

Study design

The study was performed during two 3-month periods (September-November) in 1986 and 1987, to avoid seasonal influences. During their stay in hospital, the children were not allowed to leave the ward. Fixed times were set for meals and sleeping. Three days before and during PEFR measurements at home, and three days before admission to hospital, and again three days before the exercise day, all medication was withheld.

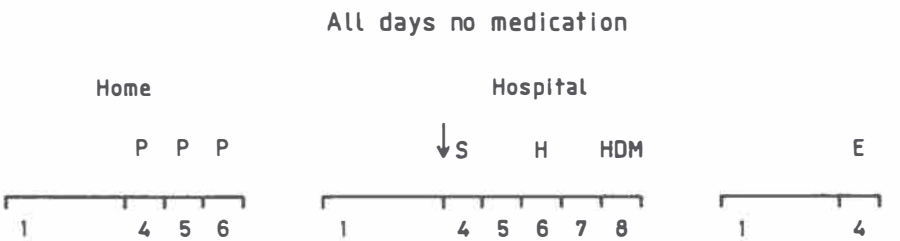


Figure 2.1: Study design of the two groups of patients. P=PEFR, S=spirometry, H=histamine inhalation provocation, HDM=house dust mite provocation, E=exercise test. The arrow indicates the first day of admission.

Patient selection was based on PEFR amplitude, measured on three consecutive days at home. PEFR measurements were performed at home on days 4, 5, and 6, at 08.00, 12.00, 16.00, 20.00, 24.00, 04.00, and 08.00 hours.

On day 4, the first day of admission, measurements were performed every 4 hours during 24 hours. Times of measurement were 08.00, 12.00, 16.00, 20.00, 24.00, 04.00, and 08.00 hours. Each set of measurements contained, in chronological order, electrocardiogram (ECG) recording and FEV_1 measurements. With intervals of 4 hours,

starting at 08.00 hours, urine samples were collected for the determination of free catecholamines and N^{τ}-methylhistamine. After this day, the children from the control group were dismissed from hospital.

On day 6, histamine inhalation challenge tests (see histamine inhalation provocation) were performed at the same points in time as on day 4.

On day 8, HDM inhalation provocations were performed at 08.00 hours (see HDM provocation). FEV_1 values were measured every 2 hours thereafter until and up to 20.00 hours. With intervals of 2 hours, starting at 08.00 hours, urine samples for the determination of the same excretion products as mentioned above, were collected until 20.00 hours.

Exercise tests (see exercise) were carried out six weeks after the HDM inhalation by the patients and their controls, in order to avoid carry-over effects of the allergen inhalation (see exercise).[1] The study protocol of the exercise day was the same as during the HDM day.

When, at 08.00 hours, FEV_1 values of days 6, 8, and the exercise day, differed more than 15% from the 08.00 hours value on day 4, the children were excluded from the study.

Pulmonary function measurements

PEFR measurements were performed with a mini-Wright peak flow meter. PEFR measurements were performed in upright position.

Spirometry was performed with a water-sealed spirometer (Lode, the Netherlands). Normal values from Zapletal and co-workers were used.[2]

The best of three efforts was used for statistical analysis.

Histamine inhalation provocation

After performing FEV_1 measurements for a baseline value, PC_{20} histamine was measured by inhalation of aerosols with increasing concentrations of the agent. The solutions were nebulized with a gauged DeVillbiss 646 nebulizer. The nebulizer was directly attached to an inspiratory-expiratory valve box.[3] The output of the nebulizer was 0.13 ml/min. The particle size was smaller than 4 μm[3,4]

The histamine acid phosphate concentrations ranged from 0.03 mg/ml to 16 mg/ml, concentration steps being 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, and 16 mg/ml. Each concentration was inhaled for 2 minutes during tidal breathing. The first aerosol delivered was phosphate buffered saline. FEV_1 values were measured 30 and 90 seconds after the end of each inhalation step. The inhalations were stopped as soon as the fall in FEV_1 had reached 20% of baseline FEV_1. The exact PC_{20} was calculated from the log-dose response curve.[5]

House dust mite provocation

The provocation with HDM was performed by inhalation of increasing concentrations of the agent. The solutions were nebulized with a gauged DeVillbiss 646 nebulizer. Nebulizer output was 0.13 ml/min. HDM extract (Diephuis, the Netherlands) was dissolved in phosphate buffered saline. All provocations were performed with mites from the same batch. The concentrations ranged from 80-10,000 biological units (BU)/ml. The concentration steps were 80 BU, 400 BU, 2000 BU, and 10,000 BU. Each concentration was inhaled for 1 minute. After performing baseline FEV_1 values, the first aerosol delivered was phosphate buffered saline. FEV_1 values were measured 30 and 90 seconds, and 15 minutes after the end of each inhalation step.

In adults 5-fold dose increases of HDM a fall of more than 20% from baseline FEV_1 are known to cause discomfort and dyspnea.[6] Therefore the inhalations in our study were stopped as soon as the fall in FEV_1 had reached 15% of baseline.

Electrocardiogram recording and measurement of vagal activity

Direct measurement of parasympathetic activity in humans is not ethically feasible, because an operation is required. Changes in heart rate and sinus arrhythmia gap may, however, reflect vagal tone.[7,8]
Standard ECG lead II on a Cardiostat 701 (Siemens, West Germany) was used for ECG recording. To measure vagal activity indirectly, we used the method described by Kallenbach et al.[9], in which breathing frequency was modified from 6 to 10 per minute to correct for age.

With the children in semirecumbent position and breathing deeply at a rate of 10 breaths per minute, the ECG was recorded for 60 seconds. The duration of each respiratory cycle was 6 seconds. The seconds were counted aloud by one of the investigators who ensured that breathing was continuous. Forced breaths and maximum respiratory manoeuvres were avoided because of the possibility of provoking bronchospasm in the asthmatic children.[10] From each ECG strip we selected and measured the longest and shortest R-R interval and converted the results from milliseconds to beats per minute. The magnitude of the SAG was expressed as the mean difference between the maximum and minimum heart rate per minute.[11]

Exercise

Exercise was performed on a Power jog treadmill (Sport Engineering Limited, United Kingdom) according to the standardized method of Godfrey.[12] It consisted of six minutes running with a speed and slope necessary to achieve a heart frequency of 180 beats or more per minute. Starting speed 8 km/hour; starting slope 6°. Spirometry was performed 1, 3, 5, 10, 15, 30, and 60 minutes after the end of the challenge.

Chemical analysis

Noradrenaline and adrenaline

Urine samples for the determination of free catecholamines were collected in polyethylene containers, with 50 mg of sodium metabisulfite as antioxidant. After the collection period (4 hours), hydrochloric acid (0.1 N) was added, leadig to a pH of approximately 3. Samples were stored at -20° C until analysis. Analysis was performed by HPLC with electrochemical detection as described by Westerink et al.[13] with the additional use of dihydroxy-benzylamine as internal standard. This procedure resulted in a coefficient of variation of 2.0% for noradrenaline, and for adrenaline of 4.6% (n = 10). These urinary catecholamine levels are closely related to the plasma catecholamine levels during the preceding hours.[14]

N^{τ}-methylhistamine

In the same urine sample as described above, N^{τ}-methylhistamine, a representative excretion product of endogenously produced histamine was determined by isotope dilution mass spectrometry.[15,16]

One day before, and during the study, the participants avoided foods known or suspected to contain histamine. The samples from the individual subjects were analysed in the same series.

Statistical analysis

Comparison between inter-individual variations was carried out by Student's t-test for unpaired observations. Comparison of intra-individual observations was carried out by Student's t-test for paired observations.

For statistical analysis, logarithmic transformation of the histamine PC_{20} value was used.

Unless stated otherwise, all values are expressed as mean ± Standard Error of the Mean (SEM).

The data of a group at a certain point in time were compared with those of another group at the same point in time. Values of group I were compared with those of group II and the controls. Data of group II and the control group were also compared.

REFERENCES

1. Cockroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in non-allergic bronchial reactivity. Clin Allergy 1977;7:503-13.
2. Zapletal A, Samanek M, Paul T. Lungfunction in children and adolescents. Methods, reference values. In: Zapletal A. Progress in respiration research. Vol. 22. Basel: Karger, 1987:114-218.
3. Juniper EF, Syty-Golda M, Hargreave FE. Histamine inhalation tests: inhalation of aerosols via a face mask versus a valve box with mouth piece. Thorax 1984;39:556-7.
4. Sterk PJ, Plomp A, Crombach MJJS, Van de Vate JF, Quanjer PH. The physical properties of a jet nebulizer and their relevance for the histamine provocation test. Bull Eur Physiopath Respir 1983;19:27-36.
5. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. Clin Allergy, 1977;72:35-43.
6. De Monchy JGR. The late allergic reaction in bronchial asthma. The Netherlands, Groningen: University of Groningen 1986, 183 pp. Dissertation.
7. Clarke JM, Hamer J, Shelton JR, Venning GR. The rhythm of the normal human heart. Lancet 1976;II:508-12.
8. Carruthers H, Taggart P. Vagotonicity to violent films and television programmes. Br Med J 1973;3:384-9.
9. Kallenbach JM, Webster T, Dowdeswell R, Reinach SG, Scott Millar RN, Zwi S. Heart rate control in asthma. Evidence of parasympathetic overactivity Chest. 1985;87:644-8.
10. Bennett T, Farquhar IK, Hosking DJ, Hampton JR. Assesment of methods for estimating autonomic control of the heart in patients with diabetes mellites. Diabetes 1978;27:1167-74.
11. Ewing DJ, Borsey DQ, Bellavere F, Clarke BF. Cardiac autonomic neuropathy in diabetes: Comparison of measures of R-R interval variation. Diabetologia 1981;21:18-24.
12. Godfrey S. Exercise testing in children. London: W.B. Saunders Co.Ltd, 1974.
13. Westerink BHC, Bosker FJ, O'Hanlon JS. Use of Alumina, Sephadex G10, ion exchange columns to purify samples for the determination of epinephrine, norepinephrine, dopamine, homovanillaic acid, and 5-hydroxy-indolacetic acid in urine. Clin Chem 1982;69:251-8.
14. Postma DS, Keyzer JJ, Koëter GH, Sluiter HJ, De Vries K. Influence of parasympathetic and sympathetic nervous system on nocturnal bronchial obstruction. Clin Sci 1985;69:251-8.
15. Keyzer JJ, Wolthers BG, Muskiet FAJ, Kauffman HF, Groen A. Determination of N^{τ}-methylhistamine in plasma and urine isotope dilution mass fragmentography.

Clin Chim Acta 1981;113:165-7.
16. Keyzer JJ, Wolthers H, Breukelman H, Van der Slik W, De Vries K. Determination of N^{τ}-methylhistamine in urine by gaschromatography using nitrogen-phosphorus detection. J of Chromatography 1983;275:261-9.

CHAPTER 3

THE REPRODUCIBILITY AND AGREEMENT OF THREE INDICES OF AIRWAY RESPONSIVENESS TO HISTAMINE IN ASTHMATIC CHILDREN

W.M.C. van Aalderen,[1] J. Gerritsen,[1] G.H. Koëter,[2]
L.Th. v.d. Weele,[3] D.S. Postma,[2] K. Knol.[1]

From the department of 1: Pediatrics and 2: Pulmonology of the University Hospital Groningen, and 3: the Computing Centre, University of Groningen, the Netherlands

Pediatric Pulmonology. In press.

ABSTRACT

In 12 asthmatic children, aged 8-14 years, we investigated the possibility of using a provocation concentration of histamine (PC) causing a 10% or 15% fall in baseline forced expiratory volume in 1 second (FEV_1), instead of a PC_{20} histamine, in the assessment of bronchial hyperresponsiveness. Inhalation challenge tests were performed on days 6 and 7 after withdrawal of medication. PC_{10}, PC_{15}, and PC_{20} were calculated from the dose response curves.

Reproducibility for PC_{10}, PC_{15}, and PC_{20} values for both days was determined by Student's t-test. No significant differences were observed. The correlation coefficient between PC_{20} on days 6 and 7 was 0.82, between PC_{15} values on days 6 and 7 it was 0.82, and between PC_{10} values on days 6 and 7 it was 0.76. The correlation coefficient between PC_{20} values on day 6 and PC_{15} values on day 7 was 0.84, and between PC_{20} values on day 6 and PC_{10} values on day 7 it was 0.82. All correlations were significant ($p<0.01$).

The predictive value of PC_{20} for PC_{10} and PC_{15} was determined by a least-squares regression line with 95% confidence intervals. Neither on day 6, nor on day 7, could any significant differences be observed in variances between PC_{10}, PC_{15}, and PC_{20}.

Our data show that both a PC_{10} and a PC_{15} can be used in the assessment of the degree of bronchial hyperresponsiveness in asthmatic children.

INTRODUCTION

Inhalation challenge tests with histamine are commonly used to assess the severity of increased bronchial responsiveness in asthmatic patients. Airway responsiveness to inhaled histamine is usually expressed as the provocation concentration producing a 20% fall (PC_{20}) in forced expiratory volume in one second (FEV_1). A disadvantage of this method is that a fall in FEV_1 of 20% or more after histamine inhalation produces dyspnea, chest tightness and wheezing.[1] In the present study, inhalation provocation tests with histamine were carried out on 2 consecutive days in a group of well-controlled asthmatic children. The results of the inhalation challenge tests with histamine were expressed as provocation concentrations producing a fall in FEV_1 of 10, 15, and 20%. We investigated whether a PC_{10} and PC_{15} histamine are as reproducible as a PC_{20} histamine.

PATIENTS AND METHODS

Patients

Eight boys and four girls with allergic asthma, aged 8 to 14 years (mean ± standard deviation: 11.7 ± 1.9), participated in this study. All children had a history of episodic wheezing on exposure to allergens and non-allergic stimuli. All children were characterized by an increased total IgE and showed at least an increased specific IgE to house dust mite. The children had a FEV_1, as a percentage of the predicted value, of > 70%. All children showed an increased bronchial responsiveness to inhaled histamine, defined as a histamine PC_{20} below 16 mg/ml (see chapter 2, histamine inhalation provocation).

Symptoms were well-controlled with maintenance medication including inhaled corticosteroids, sodium cromoglycate and, when needed, beta-2-adrenergic drugs. The children had not suffered from respiratory tract infections for at least three months prior to the study and had not used oral corticosteroids for at least six months prior to the study.

Informed consent from all children and their parents was obtained. The study was performed with approval of the Medical Ethics Committee of the University of Groningen.

Study design

Six days before the challenge tests were performed, all maintenance medication was withheld. On day 4 after withdrawal, the children were admitted to hospital. On that day FEV_1 values were measured at 08.00 hours. The children were not allowed to leave the ward.

On days 6 and 7 after withdrawal of the medication, histamine inhalation provocation tests were performed at 08.00 hours. If baseline FEV_1 values on days 6 and 7 differed more than 15% from the corresponding values on day 4, the children were excluded from the study.

Statistical analysis

From the dose-response curves, provocation concentrations of histamine, producing a 10%, 15%, and 20% fall in FEV_1 from baseline values (PC_{10}, PC_{15}, and PC_{20}), were calculated by linear interpolation between the two points before and after the PC was achieved. For statistical analysis all PC values were 10-logarithmically transformed.

To assess the intra-individual variation in baseline FEV_1 values, the difference between the highest and the lowest value of all three efforts was calculated. These

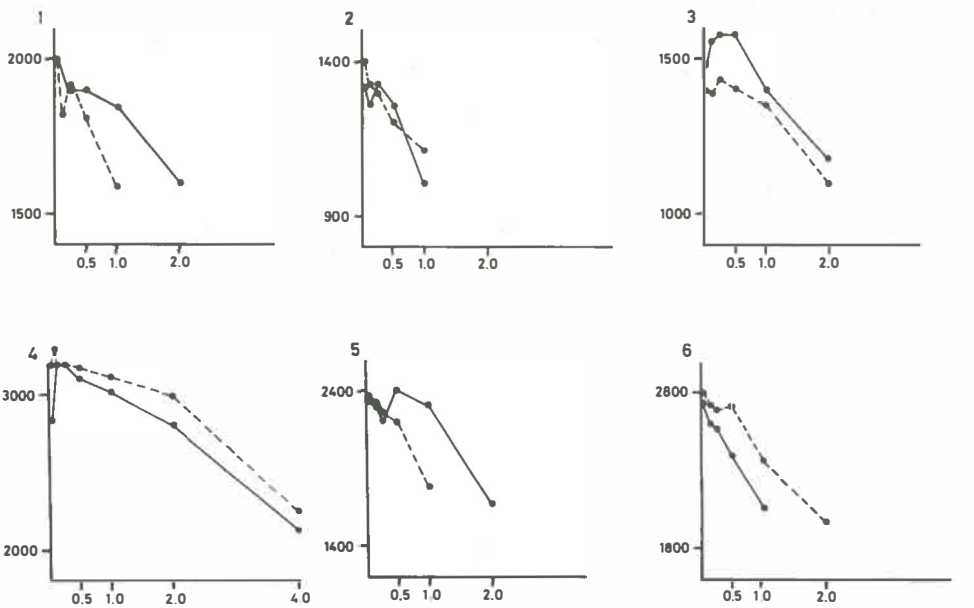
values are expressed as a percentage of the best value (mean ± standard deviation).

Reproducibility was determined by calculating the mean and SD of differences for PC_{10}, PC_{15}, and PC_{20} values. Student's t-test for paired observations was used to compare PC_{20}, PC_{15}, and PC_{10} values during the two days.

The agreement between PC_{20}, PC_{15}, and PC_{10} values on days 6 and 7 was assessed by calculation of a least-squares regression line with 95% confidence intervals and by determining a product moment correlation coefficient. The agreement between PC_{20} values on day 6, and PC_{15} and PC_{10} values on day 7, was assessed by calculation of a predictive value of PC_{20} for PC_{20}, PC_{15}, and PC_{10} respectively, by determining a product-moment correlation coefficient and a least-squares regression line with 95% confidence intervals. Since PC_{10}, PC_{15}, and PC_{20} values represent different parts, with different slopes, on the histamine dose response curve, the Bartlett statistic was applied to test the homogeneity of variances of PC_{10}, PC_{15}, and PC_{20}.

RESULTS

Of the initial 14 children who participated in the study, two were excluded because of a progressive decrease of VC and FEV_1 values on the histamine inhalation provocation days of more than 15%, compared with the FEV_1 value obtained on admission. The individual histamine dose-response curves of the remaining 12 children are shown in figure 3.1. The logarithmically transformed PC values of the children on both provocation days (day 6 and 7) are presented in table 3.1.



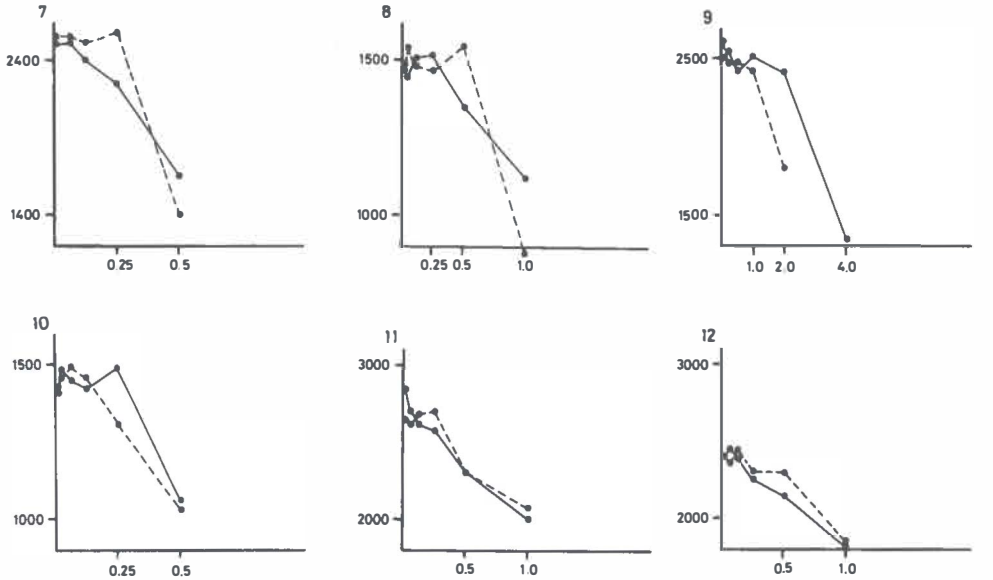


Figure 3.1: Individual histamine dose-response curves of the 12 patients on day 6 (drawn line) and day 7 (interrupted line). The X-axis represents the inhaled histamine concentrations in mg/ml, and the Y-axis represents FEV_1 values.

TABLE 3.1. ^{10}Logarithmically transformed PC_{20}, PC_{15}, and PC_{10} values of histamine of both provocation days.

	$^{10}\log PC_{20}$		$^{10}\log PC_{15}$		$^{10}\log PC_{10}$	
pat. no.	day 6	day 7	day 6	day 7	day 6	day 7
1	0.30	-0.01	0.18	-0.12	0.08	-0.28
2	-0.05	-0.03	-0.13	-0.25	-0.20	-0.43
3	0.30	0.27	0.22	0.22	0.13	0.13
4	0.55	0.50	0.50	0.44	0.43	0.38
5	0.23	-0.05	0.18	-0.13	0.12	-0.21
6	-0.11	0.17	-0.24	0.00	-0.39	-0.11
7	-0.45	-0.44	-0.51	-0.46	-0.60	-0.51
8	-0.09	-0.12	-0.19	-0.15	-0.31	-0.19
9	0.44	0.19	0.41	0.10	0.37	0.02
10	-0.30	-0.32	-0.37	-0.42	-0.41	-0.54
11	-0.25	-0.04	-0.41	-0.19	-0.55	-0.35
12	-0.09	-0.04	-0.19	-0.10	-0.32	-0.18

Intra-individual variation in baseline FEV1 values

The intra-individual variation in baseline FEV_1 values of all provocation tests was 3.3 ± 2.2%.

Reproducibility of PC_{10}, PC_{15}, and PC_{20} values

Differences of PC_{10}, PC_{15}, and PC_{20} values are presented in table 3.2 (mean ± SD). No significant differences were observed when PC_{10}, PC_{15}, and PC_{20} values on both days were compared (t values for PC_{10}, PC_{15}, and PC_{20} were 0.784, 0.731, and 0.600, respectively, for all values $p > 0.05$).

TABLE 3.2. Reproducibility of PC_{10}, PC_{15}, and PC_{20} values.

	PC_{10}	PC_{15}	PC_{20}
X	0.051	0.042	0.033
SD	0.226	0.191	0.179
t	0.792	0.756	0.644

The values show that a PC_{10}, a PC_{15}, and a PC_{20} are approximately equally reproducible. Mean (X) and standard deviation (SD) of the differences of PC_{10}, PC_{15}, and PC_{20} values between both provocation days.
t: t-value of Student's t-test.

Agreement between PC_{20} and PC_{10}, and PC_{20} and PC_{15}

The product-moment correlation coefficient of PC_{20} and PC_{15} values on days 6 and 7 was 0.82, and between PC_{10} values on both days 0.76.

The product-moment correlation coefficient of PC_{20} values on day 6 and PC_{15} values on day 7 was 0.84, and between PC_{20} values on day 6 and PC_{10} values on day 7 was 0.82. All correlations were significant ($p<0.01$). The least-squares regression lines are presented in Figure 3.2.

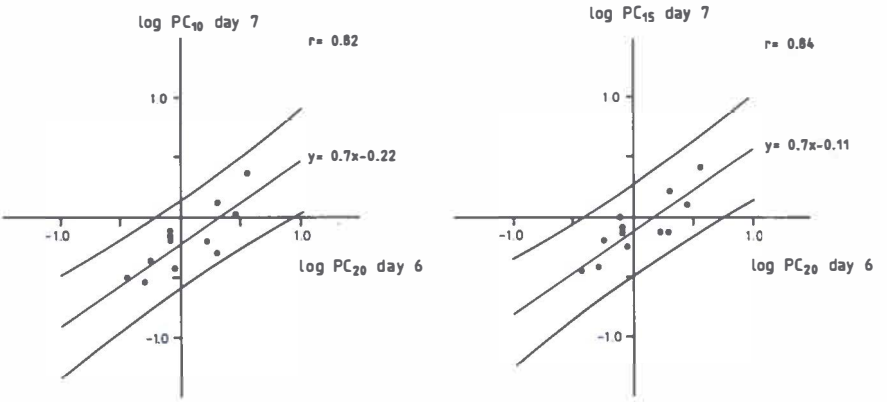


Figure 3.2: Least-squares regression line with 95% confidence intervals for PC_{20} histamine values on day 6 versus PC_{15}, and PC_{10} histamine values on day 7.

Homogeneity of variances of PC_{10}, PC_{15} and PC_{20}

On day 6, variances of PC_{10}, PC_{15}, and PC_{20} values (1.36, 1.21, and 1.10, respectively) showed no significant differences ($p = 0.94$).

On day 7, no significant differences in variances of PC_{10}, PC_{15}, and PC_{20} values were observed. Variances of PC_{10}, PC_{15}, and PC_{20} were 0.85, 0.72, and 0.71, respectively ($p = 0.93$).

In 8 out of 24 (33%) of the histamine inhalation provocation tests performed, one additional concentration had to be inhaled to induce a 20% fall in FEV_1, after a 10% fall had already been achieved.

DISCUSSION

This study shows that in the assessment of bronchial hyperresponsiveness in asthmatic children, information obtained from a PC_{15} or PC_{10} is comparable to that of a PC_{20} histamine. The results suggest that a PC_{10} is sufficient to measure the degree of bronchial hyperresponsiveness in a reproducible way. In our study, the intra-individual variation in baseline FEV_1 values was not larger than 3.3 ± 2.2%. This variation is small, but measurements were performed in a small group. Eiser et al.[2] mentioned an individual variation in FEV_1 values of 8%. When a 10% fall in FEV_1 values was used to calculate a PC value, one additional provocation dose was necessary to produce a 20% fall in 33% of the performed inhalation tests.

Histamine inhalation tests can be used to evaluate the severity of asthma.[3] They

provide clinicians with objective data for the follow-up of the disease in individual patients. These tests also give an indication of the amount of drugs necessary for control of symptoms and an evaluation of the effects of maintenance treatment.[4,5] Since the introduction of histamine inhalation provocation tests, several aspects of the provocation procedure have been altered and standardized. Tiffeneau was the first to describe inhalation provocation tests using histamine and acetylcholine to produce a definite fall in pulmonary function measurements.[6] De Vries introduced a reproducible method in which the histamine aerosol was generated by a gauged Wiesbadener doppelinhaler.[7] The aerosols were inhaled during tidal breathing for 30 seconds. This method was modified by Cockcroft et al.[8] Inhalation time was prolonged to 2 minutes, which led to a better reproducibility.[9] Generation of aerosols with other devices, such as Wright or DeVillbiss nebulizers was introduced. In addition, the particle size was standardized.[10]

At first a fall in FEV_1 of 10% from baseline was accepted,[7] later a 15%, 20% and even a 30% fall were used to indicate increased bronchial hyperresponsiveness in asthmatic patients.[1,11,12] Although dyspnea due to histamine inhalation generally does not last longer than 1 hour,[13] and is easily reversed by beta-2-adrenergic drugs, some patients may experience the procedure as troublesome; they may complain about side-effects such as throat irritation, cough, flushing, and headache.[1] Moreover, the incidence of side-effects considered acceptable in the community may be lower than the incidence for subjects in a laboratory. A lower dose of histamine is, therefore, to be preferred.[14]

The choice of a 20% fall in baseline FEV_1 is arbitrary.[14,15] Neyens et al.[11], who compared various methods, using different techniques of analysis of the dose-response relationship, concluded that a 15% decrease in FEV_1 was a suitable criterion for the provocation dose (PD) defined as the threshold, and that other criteria such as PD_{20} and PD_{25} offered no advantages over a PD_{15}. Chinn et al.[14] showed in a large community survey that PD_{10} and PD_{20} had similar repeatability. Our results are in agreement with these observations. The PC_{15}, and even the PC_{10}, on both measurement days show a high degree of correlation with the PC_{20}.

This study does not provide an answer to the question whether a PC_{10} histamine is suitable for the determination of the cut-off between asthmatic and normal. Recently, in a large population Rijcken et al.[16] used a PC_{10} histamine as being discriminative between asthmatic and normal. Yet, more research to determine the specificity of the PC_{10} histamine in diagnosing asthma still needs to be done.

The fact that PC_{20}, PC_{15}, and PC_{10} are approximately equally reproducible, and the fact that no significant differences in variance between PC_{20}, PC_{15}, and PC_{10} values were observed, indicate that a 10% fall in FEV_1 after histamine provocation is sufficient to estimate bronchial hyperresponsiveness in asthmatic children.

REFERENCES

1. Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and metacholine. Thorax, 1978; 33:705-10.
2. Eiser NM, Kerrebijn KF, Quanjer Ph H. Guidelines for standardization of bronchial challenges with (nonspecific) bronchoconstricting agents. Bull Europ Physiopath Resp, 1983;19:495-541.
3. Hargreave FE, Ryan G, Thomson MC, O'Byrne PM, Latimer K, Juniper EF, Dolovich J. Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significances. J Allergy Clin Immunol, 1981;68:345-7.
4. Kerrebijn KF, Van Essen-Zandvliet EEM, Neijens HJ. Effect of long-term treatment with inhaled corticosteroids and beta-agonists on the bronchial responsiveness in children with asthma. J Allergy Clin Immunol, 1987;79:653-9.
5. Kraan J, Koëter GH, Mark van der Th W, Sluiter HJ, De Vries K. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with antiasthmatic drugs in patients with allergic asthma: a comparison between budesonide and terbutaline. J Allergy Clin Immunol, 1985;76:628-36.
6. Tiffeneau R. Hypersensibilité cholinergo-histaminique pulmonaire de l'asthmatique. Acta Allergol, 1958; suppl.5, 187-221.
7. De Vries K, Goei JT, Booy-Noord H, Orie NGM. Changes during 24 hours of the lungfunction and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. Int Arch Allergy, 1962;20:93-101.
8. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. Clin Allergy, 1977;7:235-43.
9. Cockcroft DW, Berscheid BA. Standardization of inhalation tests: dose vs concentration of histamine. Chest, 1982;82:572-5.
10. Ryan G, Dolovich MB, Obminsky G, Cockcroft DW, Juniper EF, Hargreave FE, Newhouse MT. Standardization of inhalationprovocation tests: influence of nebulizer output, particle size and method of inhalation. J Allergy Clin Immunol, 1981;67:156-61.
11. Neijens HJ, Hofkamp PM, Degenhart HJ, Kerrebijn KF. Bronchial responsiveness as a function of inhaled histamine and the methods of measurements. Bull Europ Physiopath Resp, 1982;18:427-38.
12. Platt-Mills TAE, Mitchell EB, Nock P, Tovey ER, Moszoro H, Wilkins SR. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. Lancet, 1982;II:675-7.
13. Gerritsen J, Koëter GH, Akkerboom J, Knol K. Recovery from histamine challenge in asthmatic children. Clin Allergy, 1987;17:119-26.
14. Chinn S, Britton JR, Burney PGJ, Tattersfield AE, Papacosta AO. Estimation and

repeatability of the response to inhaled histamine in a community survey. Thorax 1987;42:45-52.
15. Cropp GJA, Berstein IL, Boushey HA, Hyde RW, Rosenthal RR, Spector SL; Townley RG. Guidelines for bronchial inhalation challenges with pharmacologic and antigenic agents. ATS news; Spring 1980:11-9.
16. Rijcken B, Schouten JP, Weiss ST, Speizer FE, Van der Lende R. The relationship between airway resonsiveness to histamine and pulmonary function level in a random population sample. Am Rev Resp Dis 1988;137:826-32.

CHAPTER 4

THE EFFECT OF REDUCTION OF MAINTENANCE TREATMENT ON CIRCADIAN VARIATION IN PEAK EXPIRATORY FLOW RATE VALUES IN ASTHMATIC CHILDREN

W.M.C. van Aalderen,[1] D.S. Postma,[2] G.H. Koëter,[2] K. Knol.[1]

From the department of 1: Pediatrics and 2: Pulmonology of the University Hospital Groningen, the Netherlands.

Acta Paediatrica Scandinavica 1988;77:269-274.

ABSTRACT

We investigated in well-controlled asthmatic children whether it is possible to predict, by measuring daytime forced expiratory volume in one second (FEV_1), by daytime peak expiratory flow rate (PEFR) values, or by drugs needed to control the disease, the decline in nocturnal PEFR values after withdrawal of maintenance medication.

FEV_1 values and PEFR values were measured in the outpatient clinic, on the last day with medication. PEFR values were then measured every four hours on days 4, 5, and 6 without medication. On the three study days seventeen children showed an amplitude in circadian PEFR values of 20% or more (group I) and nine children showed an amplitude of less than 20% (group II). Mean values ± SEM were 34.7 ± 2.1% and 10.5 ± 1.5%, respectively.

FEV_1 values were comparable in both groups. Daytime PEFR values before and after withdrawal, remained on the same level in both groups. In group I PEFR values of 24.00 and 08.00 hours on day 6 were significantly lower ($p < 0.05$) than on day 4.

The results indicate that history and daytime pulmonary function measurements alone are insufficient to assess the clinical situation, and suggest that a decrease in early morning PEFR value (08.00 hours) may be an early sign of deterioration of the disease state, after reduction of medication.

INTRODUCTION

Fluctuations in daytime PEFR values over several days may reflect the severity of asthma.[1] Besides these fluctuations, an increased variation in PEFR values can also be observed in asthmatic patients.[2]

Besides current history and physical examination most clinicians use FEV_1 measurement as a simple method for assessing the severity of the disease.

As part of a larger protocol, designed to study several aspects of circadian variation in pulmonary function, all medication was withdrawn for 6 days in a group of well-controlled asthmatic children.

During this study we investigated whether parameters such as FEV_1 expressed as percentage of the predicted value (FEV_1 % pred.), daytime PEFR values, and the drugs needed to control the disease, could be used to predict the amplitude in circadian variation after withdrawal of maintenance treatment.The second aim of the study was to find out whether pulmonary complaints, or decrease of PEFR values were to be the first signs of deterioration in the disease state.

METHODS

Patients

Forty asthmatic children, eleven girls and twenty-nine boys, aged 8 to 15 years (mean ± standard deviation: 10.9 ± 1.9) were selected from our pediatric asthma outpatients clinic to participate in this study. All children had a history of episodic wheezing on exposure to allergens or non-allergic stimuli.

Their symptoms were well-controlled with sodium cromoglycate, inhaled corticosteroids, and bronchodilators. Maintenance medication was prescribed on the clinical assessment of the disease state and on objective data such as results of a histamine inhalation provocation dose and skin tests with common allergens. All the children participating in the study showed a histamine threshold value lower than 32 mg/ml during 3 months before the study,[3] and at least one positive intracutaneous skin test to house dust mite extract (Diephuis Laboratories, The Netherlands). No oral corticosteroids were used during at least 6 months before the study.

Informed consent from all the children and their parents was obtained. The study was performed with the approval of the Medical Ethics Committee of the University Hospital of Groningen.

Study design

On the morning of the first study day a PEFR and FEV_1 measurement were carried out. The PEFR measurements were performed with a mini-Wright peak flow meter. The best of three blows was taken for statistical analysis. The FEV_1 measurements were carried out with a water-sealed spirometer (Lode).

All medication was withheld for 6 days. On days 4, 5, and 6, PEFR values were measured at home at 08.00, 12.00, 16.00, 20.00, 24.00, 04.00 and 08.00 hours. Before withdrawal of all medication, parents and children were carefully instructed to observe symptoms. The investigators were always available for consultation and advice.

Since a large amplitude of the pulmonary circadian rhythm in asthmatic patients indicates the patients at risk for death due to asthma,[4] and an amplitude of more than 20% is a useful screening test for asthma,[2] the patients were subdivided into two groups: I) with an amplitude of 20% or more, and II) with an amplitude of less than 20% on three consecutive days.

Statistical analysis

A mean PEFR value for each individual was calculated for days 4, 5, and 6. The PEFR value of each time point is expressed as a percentage of this mean value. The difference

between the highest and lowest PEFR value of the day, expressed as percentage of the highest value, is called amplitude. Comparison between intra-individual observations was carried out by Student's t-test for paired observations. Unless stated otherwise all values are expressed as a mean ± the standard error of the mean (SEM).

RESULTS

Thirty-six out of the initial forty children who participated in the study returned their PEFR values. Three children were excluded because they became dyspneic ans had to restart medication during the study. Seven children were excluded because they did not show a consistent amplitude on days 4, 5, and 6. The clinical data of the remaining twenty-six children are presented in Table 4.1. As shown in Figure 4.1, they were subdivided into 2 groups. Group I: seventeen children with an amplitude in PEFR values of 20% or more on days 4, 5, and 6; group II: nine children with an amplitude in PEFR values of less than 20% on days 4, 5, and 6.

The mean amplitudes in PEFR of days 4, 5, and 6 in group I and II were 34.7 ± 2.1% and 10.5 ± 1.5% respectively.

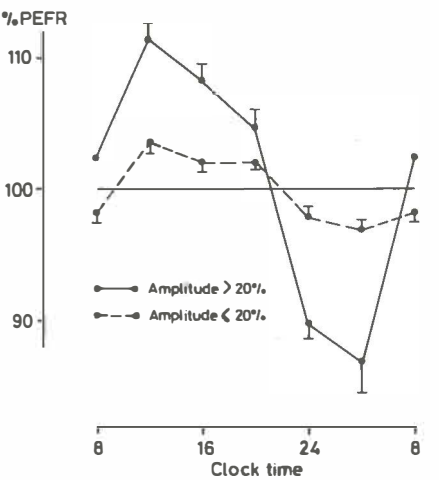


Figure 4.1:Mean circadian variation of PEFR values measured on days 4, 5, and 6 of the two groups. All values are expressed as a percentage of the calculated mean PEFR value of the day.

FEV_1 versus amplitude

The FEV_1 expressed as percentage of the predicted value (FEV_1 % pred.) of the two groups were not significantly different, mean values of group I and II being 92.3 ±2.6%, and 86.2 ± 5.1%, respectively ($p > 0.05$).

Amplitude on day 4 versus day 6

Group I showed a significant increase in amplitude when days 4 and 6 were compared (Table 4.2). The amplitudes on days 4, 5, and 6 were 31.4 ± 2.3%, 36.7 ± 2.4%, and 38.3 ± 3.0% respectively (day 4 vs. day 6, $p < 0.05$).

In group II no such phenomenon was observed.

Table 4.1. Data of 26 children participating in the study

Patient no.	Sex	Age	FEV_1 % pred.	Maintenance medication	Amplitude (%)
Group I: Amplitude ≥ 20%					
1	F	15	97	ic	34.8
2	M	9	103	sc	34.6
3	M	14	98	ic	36.3
4	M	9	88	ic	41.7
5	F	13	89	sc+t	36.7
6	M	8	105	ic	61.1
7	M	9	99	ic	30.8
8	M	12	79	sc	37.3
9	M	9	105	ic	35.5
10	M	9	72	sc	26.9
11	F	10	90	sc	26.3
12	M	9	96	sc	46.6
13	M	12	80	sc	27.2
14	M	8	93	ic	30.0
15	M	10	74	sc	28.3
16	M	12	93	ic	29.1
17	M	8	108	sc	27.6
$\bar{X}$±SEM		10.4±2.6	92.3±2.6		34.7±2.1
Group II: Amplitude < 20%					
18	M	14	87	sc	11.4
19	M	12	53	b	11.3
20	M	12	75	sc	16.5
21	F	11	98	sc	10.8
22	F	13	84	b	6.9
23	M	12	85	ic	9.4
24	F	11	105	sc	4.5
25	M	14	94	b	5.7
26	F	8	95	b	17.7
$\bar{X}$±SEM		11.9±0.6	86.2±5.1		10.5±1.5

sc=sodium cromoglycate, t=theophylline, ic=inhalation corticosteroids, b=β_2-adrenergics. FEV_1 % pred.: measured on day 1. Amplitude=mean amplitude of days 4, 5, and 6.

Table 4.2. Changes in the amplitude of circadian PEFR values in the two groups. The values are expressed as percentage of the days mean PEFR value.

		day 4	day 5	day 6
I	Amplitude ≥ 20%	31.4±2.3	36.7±2.4	38.3±3.0*
II	Amplitude < 20%	11.1±1.8	10.1±1.4	12.3±3.0

* Day 4 vs. 6, $p<0.05$ (Student's t-test).

Diurnal PEFR values

In neither groups did the diurnal PEFR values (12.00, 16.00, and 20.00 hours values) of days 4 and 6 vary significantly. Also no difference was found when daytime PEFR values of days 4 and 6 (days without medication) were compared with the daytime PEFR value of day 1 (day with maintenance medication).

Nocturnal and early morning PEFR values

In group I the 08.00 hours PEFR-value on day 6 was significantly lower as compared to day 4.

Although all seventeen children showed an amplitude of 20% or more on the three PEFR measurement days, only nine out of seventeen had a synchronized circadian rhythm with the best PEFR value performed at 16.00 hours and the worst at 04.00 hours. When in these nine children nocturnal and early morning PEFR values of day 6 were compared with day 4, a significant decrease ($p < 0.05$) was observed at 24.00 and 08.00 hours (Fig.4.2). In group II no nocturnal or early morning decline in PEFR values were observed.

Medication

In the group with an amplitude of 20% or more on days 4, 5, and 6, eight out of seventeen children (47%) used inhalation steroids, whereas only one child (11%) within the group with the consistently small amplitude of 15% or less on days 4, 5, and 6, used inhalation steroids as maintenance medication.

In the latter group four out of the nine children (19, 22, 25, and 26) used beta-adrenergics, at least once a day. The other twenty-nine children used beta-adrenergics occasionally.

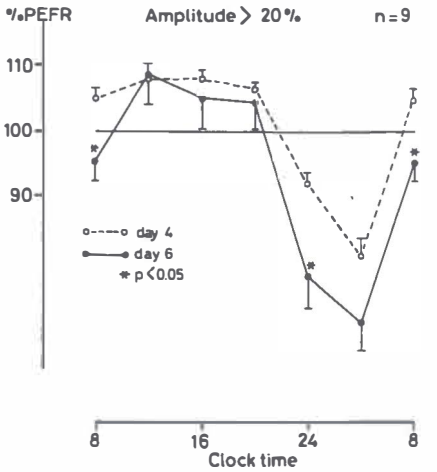


*Figure 4.2:*Circadian variation of PEFR values on days 4 and 6 of nine children from group I with a synchronized circadian variation of their PEFR values.
All values are expressed as a percentage of the calculated mean value of day 4.

DISCUSSION

The present study shows that after withdrawal of maintenance treatment in well-controlled asthmatic children large inter-individual differences in circadian PEFR amplitude can be expected.

Seventeen out of the twenty-six children showed an amplitude of more than 20% on days 4, 5, and 6 after withdrawal of the therapy. Nine children showed an amplitude of less than 20%. The morning FEV_1%pred. values obtained before withdrawal of maintenance medication in the two groups were comparable. PEFR values measured during the daytime on days 1, 4, 5, and 6 were comparable in group I and II. It was, therefore, not possible to predict which child would show a large amplitude in PEFR values by using these objective clinical measurements.

It is obvious that a clinician does not want to take the risk of suddenly stopping treatment in well-controlled asthmatic patients. Acute withdrawal of medication may accelerate the increase of amplitude of the circadian pulmonary rhythm as compared to a situation in which medication is gradually reduced. However, it was surprising that only three out of the thirty-six children had to be considered as dropouts for this reason. Although the children and parents were carefully instructed to contact us, and to restart maintenance treatment when the symptoms were troublesome, seventeen out of the thirty-six participating children (47%) in the study, showed a large decline in PEFR-values during the night, but did not restart medication. The results indicate that an increase in symptoms is underassessed by both children and parents.

Our data suggests that a nocturnal and early morning dip in PEFR values after reduction of medication is an early sign of deterioration of the disease, caused by

undertreatment. In the group with a consistent amplitude of more than 20% a significant increase in amplitude was observed when day 4 was compared with day 6. In the nine children of this group who showed a synchronized circadian rhythm, nocturnal PEFR values of day 6 were significantly decreased as compared to the nocturnal values of day 4, while day-time PEFR values remained on the same level as before. These results show that day-time PEFR measurements alone provide insufficient information about the development of the amplitude of the circadian pulmonary function. However, early morning PEFR measurements (08.00 hours), shortly after reduction of medication, help the clinicians to evaluate whether the reduced therapeutical regimen is still sufficient to control the disease.

Inhaled corticosteroids were used by eight out of seventeen patients who showed a large amplitude, while only one out of nine children with a consistently small amplitude used this kind of medication. This outcome suggests that the children with the large amplitude had already, on the basis of a clinical impression, been considered as more severe asthmatic patients. Objective parameters such as histamine threshold values performed within a period of three months before the study and FEV_1 % pred. during medication were, however, comparable in both groups.

Nocturnal bronchoconstriction may cause many complaints. Different kinds of medication, such as theophillines, corticosteroids, slow-release $ß_2$ adrenergic drugs, and ketotifen have been studied and are recommended as an adequate treatment for nocturnal dyspnea.[5-8] Corticosteroids reduce the amplitude of the pulmonary function,[5,9] and are therefore often prescribed to patients with complaints of nocturnal or early morning dyspnea. Reduction of medication may lead to an increase in amplitude of the circadian pulmonary function and thus to an increase in asthmatic complaints. In children properly treated with inhaled corticosteroids care should be taken in reducing the dose, as current medication may prevent nocturnal bronchoconstriction.

Our study shows that it is not possible to predict which asthmatic child will have a nocturnal decline in PEFR values after reduction of medication, on the basis of the FEV_1%pred. alone, obtained during medication. The history and daytime PEFR measurements after reduction of medication, also provide insufficient information about the clinical disease state.

It is advisable to evaluate the effect of a reduced therapeutical regimen on the occurrence of nocturnal bronchoconstriction by early morning (08.00 hours) PEFR measurements at home, shortly after reduction of the medication. Special attention should be paid to children who inhale corticosteroids.

REFERENCES

1. Hetzel MR, Clark TJH, Branthwaite MA. Asthma: Analysis of sudden death and ventilatory arrests in hospital. Br Med J 1977;1:808-11.
2. Hetzel MR, Clark TJH. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. Thorax 1980;35:732-8.
3. Gerritsen J, Koëter GH, Akkerboom J, Knol K. Recovery from histamine challenge in asthmatic children. Clin Allergy 1987;17:119-26.
4. Bateman JRM, Clarke SW. Sudden death in asthma. Thorax 1979;34:40-4.
5. Reinhardt D, Schuhmacher P, Fox A, Stemmann EA, Wegner F. Comparison of the effects of theophylline, prednisolone and sleep withdrawal on airway obstruction and urinary cyclic AMP/cyclic GMP excretion of asthmatic children with and without nocturnal asthma. Int J Clin Pharmacol Ther Toxicol 1980;8:399-404.
6. Postma DS, Koëter GH, Keyzer JJ, Meurs H. Influence of slow release terbutaline on the circadian variation of catecholamine, histamine and lungfunction in non-allergic patients with partly reversible airflow obstruction. J Allergy Clin Immunol 1986;77:471-7.
7. Pedersen S. Treatment of nocturnal asthma in children with a single dose of sustained-release theophylline taken after supper. Clin Allergy 1985;15:79-85.
8. Salmon MA. Multicentre study for the evaluation of ketotifen in the prophylaxis of extrinsic astma in children. Res Clin Forums 1982;4:45-50.
9. Kraan J, Koëter GH, Van der Mark ThW , Sluiter HJ, De Vries K. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with anti asthmatic drugs in patients with allergic asthma: A comparison between budesonide and terbutaline. J Allergy Clin Immunol 1985;76:628-36.

CHAPTER 5

NOCTURNAL AIRFLOW OBSTRUCTION, HISTAMINE, AND THE AUTONOMIC CENTRAL NERVOUS SYSTEM IN ALLERGIC CHILDREN WITH ASTHMA

W.M.C.van Aalderen,[1] D.S.Postma,[2] G.H.Koëter,[2] K.Knol.[1]

From the department of 1: Pediatrics and 2: Pulmonology of the University Hospital Groningen, the Netherlands.

Submitted.

ABSTRACT

In a study of two groups of nine allergic asthmatic children, one group with (group I), and one group without (group II) increased nocturnal airflow obstruction, we determined whether an imbalance in the autonomic nervous system, or inflammatory mediators like histamine, are responsible for the nocturnal increase in airflow obstruction. The results of investigations of the two groups of asthmatics were compared with the results of an age-matched control group.

Three days before, and during the study, all medication was withheld. On day 4, the day of admission, values for forced expiratory volume in one second and electrocardiogram recordings of one minute were obtained every 4 hours during 24 hours. Heart rate and sinus arrhythmia gap were used to express vagal activity indirectly. Urine was collected in periods of 4 hours between the measurements, and samples were used for the determination of adrenaline, noradrenaline, and N^{τ}-methylhistamine (a unique histamine metabolite).

In group I, overall N^{τ}-methylhistamine excretion was on a higher level compared with the values of both other groups, and was significantly higher overnight. These observations might indicate that nocturnal airflow obstruction is caused by increased release of inflammatory mediators overnight.

Endogenous orthosympathetic and parasympathetic stimulation was found to be of minor importance in the nocturnal regulation of the airway diameter.

INTRODUCTION

Nocturnal and early morning dyspnea are common symptoms in asthmatic adults and children.[1,2] In asthmatics the increase in airflow obstruction overnight is probably the result of an amplification of the normal circadian rhythm in pulmonary function,[3] but other explanations have also been offered.

One of the causes of nocturnal and early morning dyspnea might be an imbalance in the autonomic regulation of the bronchial smooth muscle tone. An increase in parasympathetic tone, via the vagal nerve, leads to bronchial smooth muscle contraction and stimulation of mucus secretion. The orthosympathetic system, by way of circulating catecholamines stimulating the β_2-receptor-adenylate cyclase complex located on the bronchial smooth muscle, is the major bronchodilating component in asthmatics. Besides leading to bronchodilatation, circulating catecholamines may also inhibit mediator release from different cell types such as mast cells, basophils etc., and in this way provide additional protection against airflow obstruction.[4]

Inflammatory reactions may also play an important role in nocturnal airflow obstruction, as suggested by the observations of Kraan et al.[5], who found that four weeks of treatment with the anti-inflammatory inhalation corticosteroid budesonide

resulted in a decrease of both the amplitude of diurnal peak expiratory flow rate (PEFR) values and of bronchial hyperresponsiveness in young adults with asthma.

In the present study, circadian variations in airflow obstruction, autonomic nervous system balance, and variations in mediator release were investigated in a group of allergic asthmatic children with and without increased airflow obstruction overnight. The aim of this study was to investigate the contribution of the above-mentioned factors to nocturnal airflow obstruction. The results of the two groups of asthmatic children were compared to those of a group matched for age.

METHODS

Study design

Three days before and during the PEFR measurements at home, as well as three days before and during the study in the hospital, all medication was withheld. The study was performed from September up to and including November in 1986 and 1987. Fixed times for meals and sleeping were set in the hospital. Before each set of measurements the children rested in supine position for 20 minutes.

On day 4, the day of admission, measurements were performed every 4 hours during 24 hours. Times of measurement were 08.00, 12.00, 16.00, 20.00, 24.00, 04.00, and 08.00 hours. Each set of measurements consisted of, in chronological order, electrocardiogram (ECG) recording and forced expiratory volume in one second (FEV_1). In periods of 4 hours, starting at 08.00 hours, urine samples were collected for the determination of free catecholamines and N^{τ}-methylhistamine.

RESULTS

For both patient groups, nine allergic asthmatic children, aged 8-15 years, were selected. The clinical parameters of all patients are presented in table 5.1. In group I, mean amplitude in PEFR values at home was $37.6 \pm 2.1\%$, and in group II $9.0 \pm 0.7\%$.

Table 5.1. Data of 18 patients participating in the study.

patient no	sex	age	FEV_1% pred.	PC_{20}	maintenance medication
group I: amplitude ≥ 20%					
1	M	9	72	2.00	ic
2	M	11	70	0.32	ic
3	M	13	75	2.00	ic
4	F	13	82	0.37	ic
5	F	13	79	0.78	sc+t
6	M	10	101	0.26	ic
7	M	12	75	1.62	ic
8	M	14	84	2.75	sc
9	M	10	75	0.37	sc
X±SEM		11.7±0.6	78.1±3.4		
group II: amplitude < 15%					
1	M	8	77	0.88	ic
2	M	10	97	1.52	sc
3	M	12	80	1.83	sc
4	F	13	90	1.70	sc
5	F	12	90	0.41	ic
6	F	10	97	6.15	sc
7	M	13	65	2.00	ic
8	M	14	95	3.65	b
9	F	10	100	4.37	sc
X±SEM		11.3±0.6	88.5±3.9		

sc=sodium cromoglycate, ic= inhalation corticosteroids t=theophylline, b=$ß_2$-adrenergic inhalation drugs. All patients occasionally used $ß_2$-adrenergic inhalation drugs. Patient 8, group II, used $ß_2$-adrenergic inhalation drugs on a regular basis.
Age is expressed in years, FEV_1 % pred. of 08.00 hours of day 4, PC_{20} is expressed in mg histamine/ml.

Pulmonary function measurements

FEV_1 values of groups I and II and of their normal controls are presented in figure 5.1. FEV_1 values of group I are significantly lower at 20.00 hours ($p < 0.05$), 24.00 hours ($p < 0.01$), and at 04.00 hours ($p < 0.001$) compared with group II. Daytime values of the two groups did not differ significantly, but were somewhat lower in group I.

At all points in time, FEV_1 values of the controls are significantly higher ($p < 0.01$) than in the two patient groups.

Parasympathetic activity

All three groups showed a circadian rhythm in heart rate (HR) with highest values during the day and lowest overnight. No significant differences were observed between the groups. Mean values ± SEM are presented in table 5.2. No circadian variation in sinus arrhythmia gap (SAG) was observed in any of the groups.

Table 5.2. Parasympathetic parameters (heart rate and sinus arrhythmia gap) of groups I, II, and the controls (C) on the time points of measurement.

	08.00	12.00	16.00	20.00	24.00	04.00	08.00
Heart rate (beats/min.)							
I :	84.9±4.0	75.8±2.5	82.4±3.7	90.9±6.1	76.2±3.9	68.7±2.4	80.9±3.6
II:	79.3±4.4	71.3±3.4	73.8±3.2	76.0±3.6	68.5±2.5	69.1±4.0	74.6±4.3
C :	83.2±4.7	75.6±2.8	78.3±2.9	81.1±3.6	75.4±3.7	72.7±3.6	82.3±4.7
Sinus arrhythmia gap							
I :	21.3±2.4	20.5±2.0	19.8±1.6	17.0±2.4	16.9±2.4	20.5±2.7	21.3±2.0
II:	23.1±2.9	24.2±3.3	26.8±2.5	23.8±3.0	22.6±1.7	22.1±2.1	23.7±1.9
C :	19.5±1.4	22.7±1.9	20.9±1.9	20.9±1.9	19.8±1.7	19.3±1.3	19.5±1.4

Orthosympathetic activity

No significant differences were observed in urine adrenaline excretion between the three groups at any point in time (Fig 5.2, upper panel). All three groups showed a comparable fall in adrenaline during the night.

Group I showed significantly higher noradrenaline excretion in the urine samples from 20.00-24.00 hours ($p<0.05$), and 24.00-04.00 hours ($p<0.01$) when compared with

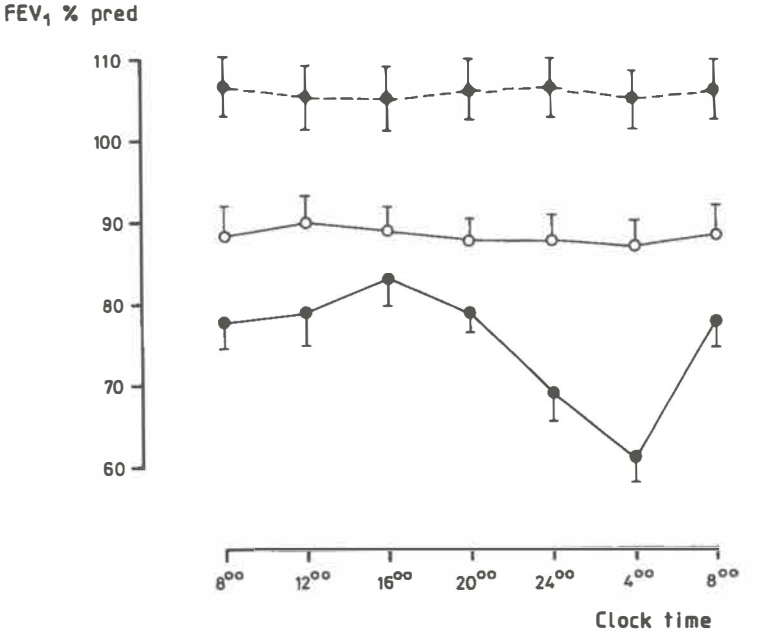


Figure 5.1:FEV_1 % predicted values of group I (-●-) and II (-o-), and controls matched for age (dotted line).

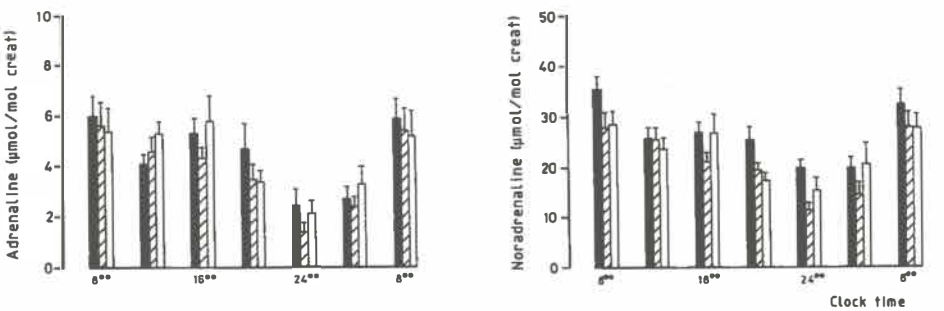


*Figure 5.2:*Urinary adrenaline (left pannel) and noradrenaline (right panel) excretion from the children in group I (black bars), in group II (striped bars), and in the control group (open bars).

these values in group II. The noradrenaline excretion in the 20.00-24.00 hours sample was also significantly higher ($p<0.05$) when compared with this value in the control group (Fig 5.2, lower panel). No significant differences were observed between the values of group II and in the control group at any point in time.

No significant correlation between individual FEV_1 values and urinary-adrenaline excretion levels was observed ($r=0.20$, and 0.02 for the values of groups I and II, respectively).

Urinary N^{τ}-methylhistamine

Group I, the group with increased airflow obstruction overnight, showed an increase in N^{τ}-methylhistamine excretion overnight. No variation was observed in N^{τ}-methylhistamine excretion in the other two groups (Fig 5.3).

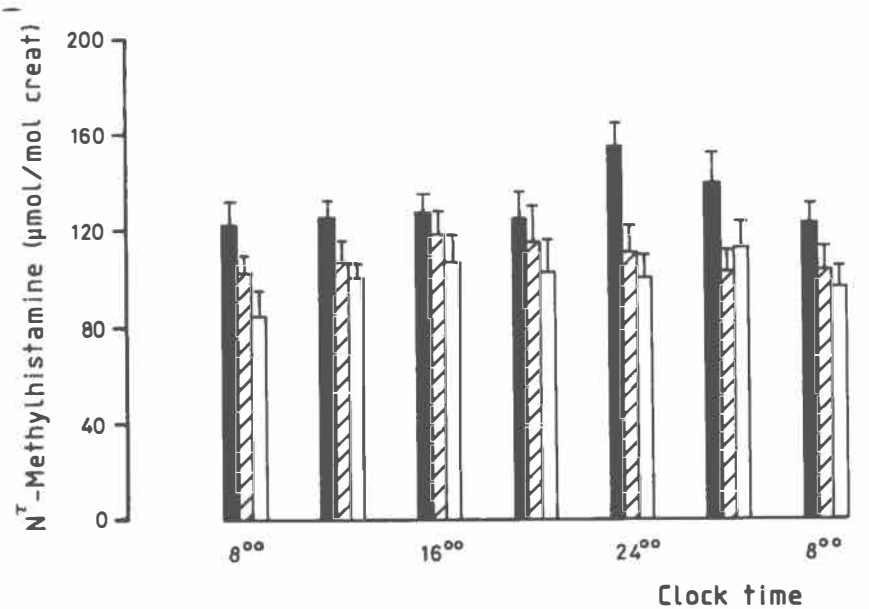


Figure 5.3:Mean change in urinary N^{τ}-methylhistamine excretion from the children in group I (black bars), group II (striped bars), and the control group (open bars).

Samples from 0.00-04.00 hours and from 04.00-08.00 hours were significantly higher in group I ($p<0.02$ and $p<0.05$ respectively) compared with the values in group II.

N^{τ}-methylhistamine values in the urine samples from 24.00-04.00 hours and from 04.00-08.00 hours were significantly higher in group I ($p<0.02$ and $p<0.05$ respectively) than in group II.

N^{τ}-methylhistamine excretion in the urine samples from 08.00-12.00 hours, 12.00-

16.00 hours, and 24.00-04.00 hours were significantly higher in group I ($p<0.05$, $p<0.05$, and $p<0.01$ respectively) when compared with these values from the control group.

Although, except for the 04.00-08.00 hours sample, the N^{τ}-methylhistamine values in group II were, higher than in the control group, differences did not attain significance.

DISCUSSION

During the night, excretion levels of N^{τ}-methylhistamine of the group with nocturnal airflow obstruction were significantly higher compared with the other two groups. During the day as well as overnight, both patient groups showed higher levels of N^{τ}-methylhistamine excretion compared with the control group, with the exception that excretion of N^{τ}-methylhistamine in the 04.00-08.00 hours sample of the control group was slightly higher as compared to group II.

These increased N^{τ}-methylhistamine levels reflect the release of histamine and possibly of other mediators from inflammatory cells. Increased mediator levels could, via irritant receptors in the larger airways, enhance the vagal tone and indirectly cause an increase in airflow obstruction. Since in the two patient groups no increase in vagal activity was observed during the day or during the night, no such indirect mechanism can explain nocturnal airflow obstruction. This suggests that the airway smooth muscle is contracted by a direct mechanism via stimulation of histamine receptors of the H_1 subtype.[6] Our observations confirm the findings of Barnes et al.[4] who observed an increase in plasma histamine during the night. In non-allergic patients with chronic obstructive lung disease and decreased FEV_1 values overnight, Postma et al.[7] observed a slight overall increase in N^{τ}-methylhistamine excretion compared with the matched controls, but no nocturnal increase. These observations indicate that at least in allergic asthmatic adults and children, mediator release is of importance to nocturnal airflow obstruction. The release of mediators is probably due to inflammatory changes in the lung occurring at night. This hypothesis is supported by the preliminary results of Martin et al.[8] In patients with nocturnal asthma they observed an increase in white blood cell count, eosinophils, and neutrophils in bronchoalveolar lavage fluid obtained at 04.00 hours compared with the 16.00 hours values.

The importance of mediator release and inflammation in nocturnal asthma is also supported by studies in which the effect of anti-inflammatory treatment was studied. Using sodium cromoglycate, Bianco et al.[9] observed a favourable change in airway resistance and pulmonary volumes in the early night and during the day. Hetzel et al.[10] observed some benefit of the same drug in terms of frequency of nocturnal wheezing and bronchodilator aerosol consumption. He did not observe an effect on nocturnal PEFR values, but this may be due to the regular use of beta-adrenergic drugs by these patients. Another argument in support of the hypothesis that nocturnal airflow

obstruction is caused by inflammation, is the observation of Kraan et al.[5] that treatment with inhaled budesonide for four weeks in young adults with asthma resulted in a decrease in the degree of bronchial hyperresponsiveness and, moreover, reduced the amplitude in diurnal PEFR values.

Why this significant difference in N^{τ}-methylhistamine excretion existed between the two patient groups in our study is not clear. Although no significant differences between groups I and II existed in daytime FEV_1 and PC_{20} histamine values, the two groups are indeed different. Daytime FEV_1 and PC_{20} values of group I are lower than those of the children from group II, and moreover, more children from group I used inhalation steroids compared with the children from group II. This indicates that the children from group I had more severe asthma, and needed more medication to prevent symptoms.

Nocturnal and early morning dyspnea in asthmatic patients is generally thought to be the result of an amplification of the normal circadian rhythm in airflow obstruction.[3]

In this study, adrenaline and noradrenaline excretion in the urine was measured as an index of adrenergic influence on bronchodilatation. In almost every sample (except for the 12.00-16.00 hours adrenaline sample), mean catecholamine excretion was higher in group I than in group II. Differences did, however, never reach a significant level. In group I noradrenaline excretion was significantly higher only in the 20.00-24.00 hours sample compared with the values of group II and the controls, and in the 24.00-04.00 hours sample it was significantly higher compared with the values in group II. These observations indicate that the ß-adrenergic system is insufficient to prevent nocturnal dyspnea.

The data of Barnes et al.[4] were confirmed in our study, a significant fall in adrenaline plasma levels overnight was observed in both asthmatics and normal subjects. In contrast to his study, we did not observe a significant correlation between FEV_1 values and urinary catecholamine values in individuals at any point in time.

Increase of parasympathetic activity overnight may be yet another cause of nocturnal airflow obstruction. The mean HR and SAG values decreased during the night, but the values were comparable in the three groups. The decrease in SAG values overnight in all three groups seems to suggest that vagal activity is even lower than during the day. All our mean SAG values were lower compared with the asthmatic adults decribed by Kallenbach et al.[11] Our observations suggest that an increased vagal tone is not of major importance in the etiology of nocturnal airflow obstruction in asthmatic children. It is possible that the influence of the parasympathetic system becomes important only at an older age, since an increase in magnitude of the SAG at night was observed by both Postma et al.[7] in non-allergic adults with chronic obstructive lung disease, and Kallenbach et al.[11] in asthmatic adults. This age-dependent generation of circadian rhythmicity has also been observed for other rhythms.[12]

The finding that parasympathetic activity in asthmatic children is not an important

factor in nocturnal airflow obstruction is indirectly supported by the observation that ipratropium bromide, a parasympathetic blocking drug, is not, or only at high dosages, effective against nocturnal dyspnea.[13,14] It seems, however, to have a beneficial effect in adults with asthma.[15]

In conclusion, our results suggest that in allergic asthmatic children histamine and possible other inflammatory mediators seem to be the determinant for nocturnal airflow obstruction. We hypothesize that environmental factors, and especially exposure to allergens during the day, induce inflammatory processes in the lung overnight. We conclude moreover, that endogenous catecholamine production does not seem to be sufficient to prevent the fall in nocturnal FEV_1 values, and that parasympathetic activity does not play a role of importance in the development of nocturnal airflow obstruction in allergic asthmatic chidren.

REFERENCES

1. Connoly CK. Diurnal rhythms in airway obstruction. Br J Dis Chest 1979;73:357-66.
2. Van Aalderen WMC, Postma DS, Koëter GH, Knol K. The effect of reduction of maintenance treatment on circadian variation in peak expiratory flow rate values in asthmatic children. Acta Pediatr Scand 1988;77:269-74.
3. Hetzel MR, Clark TJH. Comparison of normal and circadian rhythms in peak expiratory flow rate. Thorax 1980;35:732-8.
4. Barnes P, FitzGerald G, Brown M, Dollery C. Nocturnal asthma and changes in circulating epinephrine, histamine and cortisol. New Eng J Med 1980;303:263-7.
5. Kraan J, Koëter GH, Van der Mark Th W, Sluiter HJ, De Vries K. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with anti-asthmatic drugs in patients with allergic asthma: a comparison between Budesonide and Terbutaline. J Allergy Clin Immunol 1985;76:628-36.
6. Finney MJB, Karlsson JA, Persson CGA. Effects of bronchoconstrictors and bronchodilators on a novel human small airway preparation. Br J Pharmacol 1985;85:29-36.
7. Postma DS, Keyzer JJ, Koëter GH, Sluiter HJ, De Vries K Influence of parasympathetic and sympathtic nervous system on nocturnal bronchial obstruction. Clin Sci 1985;69:251-8.
8. Martin RJ, Cicutto LC, Ballard RD, Szefler SJ. Airway inflammation and nocturnal asthma (Abstract). Am Rev Resp Dis 1988;137suppl:284.
9. Bianco S, Fumagalli G, Ghiringhelli G, Allegra L. Influence and use of the employ of Disodium Cromoglycate "DSCG" on circadian rhythms in pulmonary mechanics. Revue de l'institute d'hygiene des mines. 1973;28:11-21.
10. Hetzel MR, Clarke JH, Gillam SJ, Isaac P, Perkins M. Is Sodium Cromoglycate effective in nocturnal asthma? Thorax 1985;40:793-4.
11. Kallenbach JM, Webster T, Dowdeswell R, Reinach SG, Scott Millar RN, Zwi S. Heart rate control in asthma. Evidence of parasympathetic overactivity. Chest 1985;87:644-8.
12. Rietveld WJ. Clinical aspects of chronobiology. Introduction. In Rietveld WJ, ed. Clinical aspects of chronobiology. Baarn, the Netherlands: Bakker, 1984:5-11.
13. Sly PD, Landau LI, Olinski A. Failure of Ipratropiumbromide to modify the diurnal variation of asthma in asthmatic children. Thorax 1987;42:357-60.
14. Gaultier C, Reinberg A, Girard F. Circadian Rhythms in lung resistence and dynamic lung compliance of healthy children. Effect of two bronchodilators. Respiration Physiology 1977;31:169-82.
15. Morrison JFJ, Pearson SB, Dean HG. Parasympathetic nervous system in nocturnal asthma. Br Med J 1988;269:1427-9.

CHAPTER 6

BRONCHIAL HYPERRESPONSIVENESS AND NOCTURNAL AIRFLOW OBSTRUCTION IN ASTHMATIC CHILDREN

Wim M.C. van Aalderen,[1] Dirkje S. Postma,[2] Gerard H. Koëter,[2] Klaas Knol.[1]

From the department of 1: Pediatrics, and 2: Pulmonology of the University Hospital Groningen, the Netherlands.

Thorax. In press.

ABSTRACT

In two groups of nine asthmatic children, with (group I) and without (group II) increased airflow obstruction overnight, we investigated whether an increase in bronchial hyperresponsiveness during the night might be the consequence of an increase in nocturnal airflow obstruction. Patient selection was based on the amplitudes in PEFR values measured every four hours on three consecutive days at home. All medication was withheld three days before, and during the measurements, at home as well as in the hospital.

The study was performed in the hospital on four consecutive days. On day 4, the first day of admission, FEV_1 values were measured at 08.00, 12.00, 16.00, 20.00, 24.00, 04.00, and 08.00 hours. On day 6, histamine inhalation provocation tests were performed at the same points in time as on day 4. The PC_{20} values in both groups I and II showed a nocturnal decrease, and were comparable at all points in time.

The results indicate that increased nocturnal bronchial hyperresponsiveness is not determined by the degree of airflow obstruction and suggest that other factors are responsible for the transient increase in airway responsiveness.

INTRODUCTION

Nocturnal and early morning dyspnea is a common symptom in one third of the patients with asthma.[1]

One of the proposed mechanisms that might explain a nightly fall in pulmonary function is a higher degree of bronchial hyperresponsiveness during the night. Such an explanation seems to be supported by inhalation provocation tests with bronchial obstructive agents during 24 hours. In the past, De Vries and co-workers, and Reinberg and co-workers observed a significant increase in bronchial hyperresponsiveness at 04.00 hours in the morning.[2,3]

In allergic patients with asthma, the degree of bronchial hyperresponsiveness as measured by PC_{20} histamine or acetylcholine,[2,3] correlates with the degree of airflow obstruction before provocation. It might therefore be expected that an increase in airflow obstruction would be associated with an increase in airway responsiveness. As both bronchial hyperresponsiveness and airflow obstruction are known to increase at night, the increase in bronchial hyperresponsiveness overnight might simply be the result of an increased airflow obstruction.

The aim of the present study was to investigate whether an increase in bronchial hyperresponsiveness during the night might be the consequence of a nocturnal decline in pulmonary function. Therefore we selected two groups of asthmatic children on the basis of PEFR measurements at home. Group I showed a decrease in pulmonary function during the night. In group II, no decrease occured. In both groups, spirometry and inhalation provocation tests with histamine were carried out every 4 hours, for 24 hours.

METHODS

Study design

To obtain groups of children with a stable amplitude in their pulmonary function, the selection was based upon PEFR measurements at home.[4] PEFR measurements were performed every 4 hours, on three consecutive days at home. Because of the influence of medication on bronchial hyperresponsiveness and the amplitude of the pulmonary function,[5,6] all medication was withheld three days before and during the measurements at home as well as in hospital. PEFR measurements were performed at 08.00, 12.00, 16.00, 20.00, 24.00, 04.00, and 08.00 hours.

The patients were assigned to 2 groups. Group I: amplitude in PEFR values ≥ 20% on three consecutive measurement days, and group II: amplitude < 15% on the three days.

The study was performed from September up to and including November in 1986 and 1987. Fixed times were set for meals and sleeping in hospital. On day 4, the first day of admission, FEV_1 values were measured. When the 24 hour amplitude in FEV_1 values in hospital was not comparable to the amplitude in PEFR values at home, the children were excluded from the study.

For PEFR values at home as well as the FEV_1 values in hospital, the best of three efforts was used for statistical analysis. On day 6, histamine inhalation provocation tests were performed. FEV_1 measurements on day 4 and histamine inhalation provocation tests on day 6 were performed at the same points in time as the PEFR measurements at home. Bronchodilators were withheld during the whole study.

RESULTS

Eighteen patients participated in the study. Group I: nine children, 7 boys and 2 girls, aged 9-14, showing an amplitude in PEFR values of more than 20% (mean ± SEM: 37.6 ± 2.1%). Group II: nine children, 5 boys and 4 girls, aged 8-14, showing an amplitude in PEFR values of less than 15% (mean ± SEM: 9.0 ± 0.7%). The characteristics of these patients are given in Table 5.1.

PEFR values at home

In group I a significant increase in amplitude was observed when day 4 was compared to day 6 ($p<0.05$); mean amplitudes on day 4 and 6 were 33.0 ± 3.2% and 45.0 ± 2.9%, respectively. The increase in amplitude was primarily caused by a decrease in nocturnal and early morning PEFR values. When the PEFR values of day 4 were compared with those of day 6, a significant decrease in PEFR values at 04.00 hours ($p<0.05$) was observed. The PEFR values at 04.00 hours on days 4 and 6 were 83.4 ± 1.2% and 74.8 ± 3.4%, respectively (Fig. 6.1, upper panel).

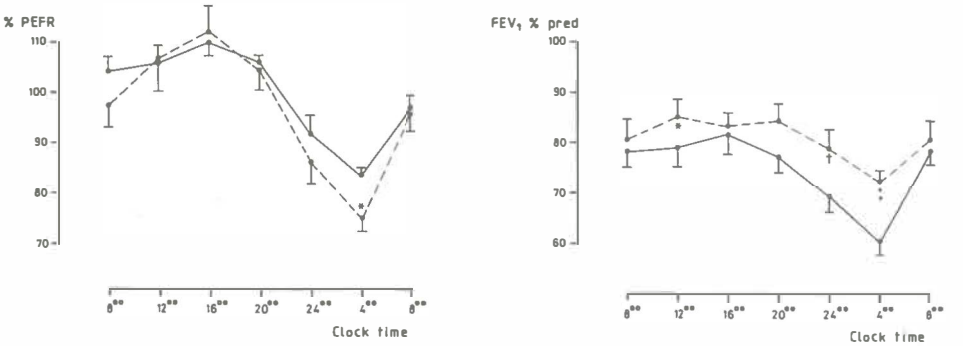


Figure 6.1:Circadian change in PEFR values at home (left panel), and circadian change in FEV_1 % pred. values in hospital (right panel) of group I.
Continuous line: day 4; interrupted line: day 6 * $p<0.05$; † $p<0.01$; ‡ $p<0.001$.

In group II no significant decrease in nocturnal and early morning PEFR values was observed. The mean amplitudes on days 4 and 6 were 8.9 ± 1.5% and 9.6 ± 1.4%, respectively.

FEV_1 values in hospital

At 08.00 hours, the mean FEV_1% pred. value in group I was 78.1±3.4%; in group II this was 88.5±3.9% ($p>0.05$, not significantly different). At 24.00 and 04.00 hours the FEV_1 % pred. values in group I were significantly lower than in group II ($p<0.01$ and $p<0.001$, respectively). In group I, FEV_1 values before histamine provocation on day 6 showed a significant increase of primarily nocturnal FEV_1 values as compared with the values of day 4 (Fig. 6.1, lower panel). In group I significant differences in FEV_1 % pred. between days 4 and 6 were observed at 12.00 hours: 79.0 ± 4.3% and 85.1 ± 3.7% ($p<0.02$), at 24.00 hours: 69.7 ± 3.9 and 79.4 ± 4.2% ($p<0.02$), and at 04.00 hours: 63.1 ± 3.0% and 72.6 ± 2.7% ($p<0.01$), respectively. No significant change in FEV_1 values from day 4 to 6 was observed in group II.

Although in group I the FEV_1 value at 04.00 hours increased during the admission. the FEV_1 value at 24.00 and 04.00 hours on day 6 were still significantly higher ($p<0.05$ and $p<0.001$, respectively) in group II.

Histamine provocation tests

Both groups showed a comparable significant decrease ($p<0.01$) in PC_{20} histamine values when 16.00 hour values were compared with the 04.00 hour value (Fig. 6.2).

When PC_{20} values in the two groups at all points in time were compared, no significant differences wereobserved (Table 6.1).

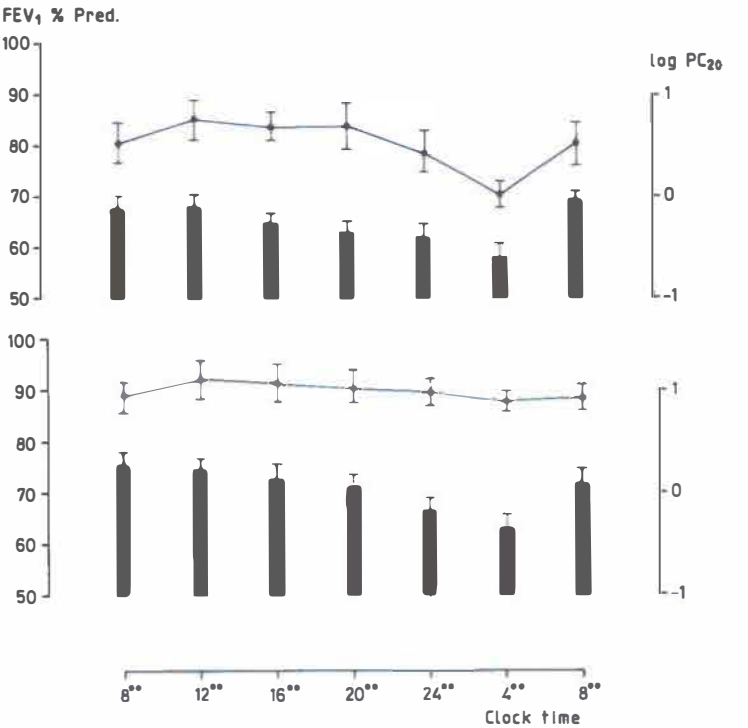


Figure 6.2:Baseline FEV_1 values (continuous lines), and ^{10}log. transformed PC_{20} values (columns) of group I (upper panel) and group II (lower panel).

Table 6.1. Geometric mean ± SEM of ^{10}logPC_{20} histamine values in mg/ml of group I and II in hospital.

	08.00 hours	12.00 hours	16.00 hours	20.00 hours	24.00 hours	04.00 hours	8.00 hours
I	-0.09 ±0.13	-0.09 ±0.12	-0.26 ±0.16	-0.36 ±0.15	-0.41 ±0.15	-0.60 ±0.17	-0.02 ±0.11
II	0.28 ±0.12	0.23 ±0.13	0.14 ±0.17	0.06 ±0.16	-0.18 ±0.12	-0.36 ±0.14	0.08 ±0.14

DISCUSSION

We investigated two groups of allergic asthmatic children, one group with, and the other without increased airflow obstruction during the night. Although the difference between groups I and II in mean FEV_1 values and mean PC_{20} values is not significant, the clinical parameters of the two groups are indeed different. Mean FEV_1 and PC_{20} values in group I are lower than in group II, and more inhaled corticosteroids were used by the children of group I, indicating that this group had more severe asthma.

Both groups showed a comparable nocturnal decrease in PC_{20} histamine values. Our data indicate that an increase in nocturnal bronchial hyperresponsiveness is not dependent on increased airflow obstruction during the night. Repeated histamine inhalation tests can cause tachyphylaxis.[7] The fact that the two PC_{20} values at 08.00 hours on days 6 and 7 from group I as well as from group II were virtually the same, showed that this did not happen in our study.

In addition, after withdrawal of maintenance medication, we observed an increase in amplitude in PEFR values at home in the group which already had a large amplitude (group I). After admission to hospital, the amplitude in FEV_1 values diminished in the same group of asthmatic children, after withdrawal of maintenance medication. In our study we did not observe this change in amplitude of the pulmonary function in group II. The results suggest that environmental stimuli are a contributing factor in the increase in amplitude in circadian pulmonary function in the patients of group I, and seem to be less relevant in group II.

De Vries and co-workers and Tammeling and co-workers were among the first to demonstrate an increased bronchial hyperresponsiveness on histamine challenge in allergic asthmatic and bronchitic patients with nocturnal dyspnea.[2,8] In these studies the airway susceptibility to histamine was greatest at 24.00 and 04.00 hours. Investigations by Reinberg et al.[3] who used acetylcholine, confirmed this circadian rhythm in bronchial hyperresponsiveness, and revealed that the nocturnal increase in bronchial hyperresponsiveness at night is a general characteristic of these patients. In these studies, however, selection criteria were not based on amplitudes in airflow obstruction during 24 hours. Therefore, these studies do not show whether an increase in bronchial hyperresponsiveness during the night is the consequence of increased airflow obstruction, or is independent of it. Our study is the first to show that an increased nocturnal hyperresponsiveness is independent of the increased airflow obstruction at night.

Ryan and associates observed a poor correlation between PC_{20} and the diurnal amplitude unless post-salbutamol values were considered.[9] The authors suggest that a PC_{20} measured in the morning may be a determinant of the diurnal amplitude in flow rates. All but two children in our study had a PC_{20} of below 4 mg/ml, but nevertheless 9 children showed large fluctuations in PEFR and FEV_1 values, and 9 showed a small

fluctuation. Ryan et al. studied a group of patients which were less hyperresponsive than the children from our group. Differences in results may also stem from a difference in study design. While our patients were investigated every 4 hours for 24 hours, Ryan et al. only performed measurements at two time points per day.

Almost invariably, bronchodilation is associated with reduced bronchial hyperresponsiveness, irrespective of whether bronchodilation occurs spontaneously,[10,11] or after drugs.[12-14] Conversely, bronchoconstriction is usually associated with increased bronchial hyperresponsiveness.[15,16] These shifts in bronchial hyperresponsiveness have led to the hypothesis that increased bronchial hyperresponsiveness is simply a reflection of changes in bronchial smooth muscle tone.[17]

Our study indicates that an increased bronchial hyperresponsiveness during the night is not determined by the degree of airflow obstruction, but is an independent feature. Conversely, mechanisms other than an increased bronchial hyperresponsiveness seem to be responsible for the nocturnal fall in FEV_1 values in asthmatic patients. Why patients with comparable, low PC_{20} histamine values at 04.00 hours do not all have the same low FEV_1 values remains unclear; some of them have comparable day and night values of FEV_1, while PC_{20} histamine values during the day are significantly higher than overnight. Other factors than bronchial hyperresponsiveness must be responsible for the modulation of the degree of airflow obstruction.

In our study FEV_1 baseline values at 04.00 hours from group I and II differed significantly ($p<0.01$), but no difference in PC_{20} values could be shown. Our observations in asthmatic children with a small amplitude (group II) are in agreement with the results of Sluiter et al.[18] in patients with emphysema, showing an increase in bronchial hyperreactivity at 24.00 and 04.00 hours, accompanied by stable FEV_1 values during day and night. An explanation of this finding may be that in this latter, supposedly non-allergic, group the airflow obstruction is already severe and, more importantly, irreversible. Another possible explanation is that circadian swings in PC_{20} values occur in all human beings.

The recognition that house dust mite faecal pellet is the source of a very potent antigen in patient with house dust mite allergy, has led to the suggestion that intensive contact with mites in beddings might precipitate airflow obstruction at night. Recently, strict exclusion of house dust mite has been shown to decrease bronchial hyperresponsiveness and morning dyspnea.[20]

In our group of children who showed a large circadian amplitude in pulmonary function (group I), we observed a significant increase in amplitude of PEFR values when they were at home without medication. The first sign of deterioration of the disease was the drop in nocturnal PEFR values, while day-time values remained normal. However, when the same children were admitted to hospital, the amplitude of FEV_1% pred. values decreased significantly. Such instability in amplitude was not measured in the children of group II, neither in the home situation, nor in hospital. It is

possible that exposure to inhaled allergens during the day leads to nightly symptoms of dyspnea due to a late asthmatic response.[21] This would suggest that allergen avoidance during the day could lead to a reduction of symptoms overnight.

We cannot account for the observation that children who already have a large amplitude, are susceptible to environmental changes. It is possible that the group with the large amplitude (group I) is more exposed to allergic and non-allergic stimuli at home, and therefore shows a more instable amplitude in pulmonary function in different environmental situations, after withdrawal of medication. Barnes et al.[22] showed an increase in nocturnal plasma levels of histamine in a group of allergic asthmatic adults with nocturnal airflow obstruction, and hypothesized that this nocturnal increase could contribute to nocturnal dyspnea. A difference in histamine production at night between our two groups could be another explanation for our observation.

In summary, we conclude that increased bronchial hyperresponsiveness during the night is not in itself responsible for nocturnal dyspnea in allergic asthmatic children, because we observed a comparable decrease in nocturnal PC_{20} values in asthmatic children both with, and without a nocturnal dip in their FEV_1% pred. values. Furthermore, we conclude that environmental factors at home, and a possible reduction of these factors in the hospital, may influence nocturnal airflow obstruction in opposite directions after withdrawal of medication.

REFERENCES

1. Connolly CK. Diurnal rhythms in airway obstruction. Br J Dis Chest 1979;73:357-66.
2. De Vries K, Goei JT, Booy-Noord H, Orie NGM. Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. Int Arch Allergy 1962;20:93-101.
3. Reinberg A, Gervais P, Morin M, Abulker C. Rythme circadien humain du seuil de la response bronchique a l'acetylcholine. Comp-Rend, Acad sci (Paris) 1971;272: 1879-81.
4. Postma DS, Koëter GH, Van der Mark Th W, Peset Reig R, Sluiter HJ. The effects of oral slow-release Terbutaline on the circadian variation in spirometry and arterial blood gas levels in patients with chronic airflow obstruction. Chest 1985;87:653-7.
5. Kraan J, Koëter GH, Van der Mark Th W, Sluiter HJ, De Vries K. Changes in bronchial hyperreactivity induced by 4 weeks of treatment with anti asthmatic drugs in patients with allergic asthma: a comparison between Budesonide and Terbutaline. J Allergy Clin Immunol 1985;76:628-36.
6. Van Aalderen WMC, Postma DS, Koëter GH, Knol K. The effect of withdrawal of maintenance treatment on circadian variation in peak expiratory fow rate values in asthmatic children. Acta Pediatr Scand 1988;77:269-74.
7. Manning PJ, Jones GL, O-Byrne P. Tachyphylaxis to inhaled histamine in asthmatic subjects. J Appl Physiol 1987;63:1572-7
8. Tammeling GJ, De Vries K, Kruyt EW, The circadian pattern of the bronchial reactivity to histamine in healthy subjects and in patients with obstructive lung disease. In: McGovern JP, Smolensky MH, Reinberg A, eds. Chronobiology in Allergy and Immunology. CC Thomas, Springfield Ill. 1977; 139-49.
9. Ryan G, Latimer KM, Dolovich J, Hargreave FE. Bronchial responsiveness to histamine: relationship to diurnal variation of peak flow rate, improvement after bronchodilator, and airway calibre. Thorax 1982;37:423-9.
10. Orehek J, Gayrard P. Les tests de provocation bronchique non specifiques dans l'asthme. Bull Europ Physiopath Resp 1976;12:565-98.
11. Geubelle F, Barlee-Harmans G, Leclercq-Foucart J. Hyperreactivity of the bronchial tree to histamine in asthmatic children and its variations. Bull Europ Physiopath Resp 1971;7:839-40.
12. Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Protective effect of drugs on histamine induced asthma. Thorax 1977;32:429-37.
13. Booy-Noord H, Quanjer PH, De vries K. Protective effect of Berotec in provocation tests using inhalation of specific allergens and histamine. Int J Clin Pharmacol 1972;4:69-72.
14. Hartley JPR, Nogrady SG. Effect of an inhaled antihistamine on exercise-induced

asthma. Thorax 1980;35:675-9.
15. Orehek J, Massari JP, Gayrard P, Grimaud C, Charpin J. Effect of short-term low level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. J Clin Invest 1976;57:301-7.
16. Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in non-allergic bronchial reactivity. Clin Allergy 1977;7:503-13.
17. Benson MK. Bronchial hyperreactivity. Br J Dis Chest 1975; 69:227-39.
18. Sluiter HJ, Orie NGM, Vegter JJM, Tammeling GJ. Emphysema and Cor Pulmonale. In: Orie NGM, Sluiter HJ, eds. Bronchitis I. Assen: Royal van Gorcum 1961.pp 233-46.
19. Tovey ER, Chapman MD, Platt Mills TAE. Mite faeces are a major source of house dust allergens. Nature 1981;289:592-3.
20. Platt-Mills TAE, Mitchell EB, Nock P, Tovey ER, Moszoro H, Wilkins SR. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. Lancet 1982;II:675-7.
21. Newman-Taylor AJ, Davies RJ, Hendrick OJ, Pepys J. Recurrent nocturnal asthmatic reations to bronchial provocation tests. Clin Allergy 1979;9:213-9.
22. Barnes P, FitzGerald G, Brown M, Dollery C. Nocturnal asthma and changes in circulating epinephrine, histamine and cortisol. N Engl J Med 1980;303:263-7.

CHAPTER 7

ADRENERGIC RESPONSE IN CHILDREN WITH AND WITHOUT NOCTURNAL ASTHMA ON EXOGENOUS STIMULI

W.M.C.van Aalderen,[1] D.S.Postma,[2] G.H.Koëter,[2]
J.G.R. de Monchy,[3] K.Knol.[1]

From the department of 1: Pediatrics, 2: Pulmonology and 3: Allergology of the University Hospital Groningen, the Netherlands.

INTRODUCTION

Nocturnal airflow obstruction in asthmatic children may be induced by both endogenous and exogenous stimuli. The nightly fall in pulmonary function in asthmatic children may, for instance, be the result of late obstructive reactions (LOR) due to allergen exposure during the day. Moreover, it has been shown that an inhalation test with allergen may lead to recurrent asthmatic attacks for many nights.[1,2] Besides challenge with allergens, exercise has also been found to produce early and late obstructive reactions (EOR and LOR, respectively).[3,4]

We hypothesized that asthmatic children with increased nocturnal airflow obstruction may suffer more frequently from LOR due to exogenous stimuli during the day than asthmatic children without increased nocturnal airflow obstruction. Therefore we investigated bronchial obstructive reaction patterns after HDM inhalation and after exercise, in asthmatic children with and without increased nocturnal airflow obstruction. Additionally, we investigated whether any differences between the two groups were caused by differences in adrenaline and noradrenaline response, or by differences in N^{τ}-methylhistamine excretion (as a representative excretion product of histamine release) during and after both stimuli. The results during and after exercise in both groups of asthmatic children were compared with the results of an age-matched group of controls.

METHODS

Study design

Three days before and during the PEFR measurements at home, as well as three days before and during the study in the hospital, all medication was withheld. The study was performed from September up to and including November in 1986 and in 1987. Fixed times for meals and sleeping were set in the hospital.

On day 4, the first day of admission, FEV_1 values were obtained every 4 hours during 24 hours. Times of measurement were 08.00, 12.00, 16.00, 20.00, 24.00, 04.00, and 08.00 hours. Before each set of measurements the children rested in supine position for 20 minutes. With intervalls of 4 hours, starting at 08.00 hours, urine samples were collected for the determination of free catecholamines and N^{τ}-methylhistamine. The values from the samples from 08.00-20.00 hours served as control values to the values obtained on both provocation days.

On day 8, HDM inhalation tests (see chapter 2, house dust mite provocation) were performed at 08.00 hours. FEV_1 values were then measured with intervals of 2 hours up to and including 20.00 hours. With intervals of 2 hours, urine samples for the determination of free catecholamines and N^{τ}-methylhistamine were collected starting at

8.00 hours until 20.00 hours.

Exercise tests (see chapter 2, exercise) were carried out six weeks after the HDM inhalation, in order to avoid carry-over effects of the allergen inhalation,[5] The patients performed an exercise test at 08.00 hours. The rest of the study protocol was the same as during the HDM provocation day.

When individual FEV_1 values at 8.00 hours on each provocation day differed more than 15% from the 8.00 hour FEV_1 value of the control day, the children were excluded from the study.

Since on the control day only 4-hourly measurements were performed, and on both provocation days measurements were performed with 2-hour intervals, the data in between two control points of measurement on the challenge day (10.00, 14.00, 18.00 hours) were compared with the preceding values on the control day.

The early obstructive reaction (EOR) is defined as a fall of 15% or more from the 08.00 hours prechallenge FEV_1 value, within the first hour after the challenge on the provocation days.

The LOR is defined as the first fall in FEV_1 of 15% or more, occurring 3-10 hours after the EOR, expressed as percentage difference between the FEV1 value at a given point in time on the provocation day and the same time of the day on the control day.

RESULTS

For both patients groups, nine allergic asthmatic children, aged 8-15 years were selected. The clinical data of all patients are presented in table 5.1.

Pulmonary function measurements

Forced expiratory volume values in 1 second (FEV_1) on the first day of admission for all three groups are presented in figure 5.1. For all points of measurement the FEV_1 values of the controls were significantly higher ($p<0.05$) than in the two patient groups.

The 08.00 hours FEV_1 value in group I was 78.1 ± 3.5%, and in group II 88.5 ± 3.9%. The difference between the groups is not statisticaly significant.

FEV_1 values after house dust mite provocation

According to the protocol all patients were challenged until they developed a fall in FEV_1 of at least 15% compared with prechallenge FEV_1 values. Mean fall in FEV_1 during the EOR of group I was 37.4 ± 5.7%, and of group II 25.2 ± 3.9%. The difference in percentage fall in FEV_1 between the two groups was not significant ($p>0.05$). The allergen dosages needed to induce an EOR (mean ^{5}log.dose ± SEM) were

for group I 3,32 ± 1,01 and for group II 3,56 ± 0,88 BU/min, respectively, the difference being not significant. Prechallenge FEV_1%pred. value in group I was 81.1 ± 2.8%, and in group II 89.6 ± 3.1% (no significant difference between groups I and II; no significant difference with control day values of both groups).

Seven out of 9 patients in group I, and 8 out of 9 in group II developed a LOR. There were no differences in percentage fall of FEV_1 during the LOR between the two patient groups. Mean values of the percentage fall for groups I and II were 37.1 ± 6.2% and 36.4 ± 3.9% respectively.

FEV_1 values after exercise

All children performed a treadmill exercise test with similar workload.[6,7] Heart rate was kept over 180 beats per minute in all children.

Prechallenge FEV_1%pred. values of groups I and II were 81.3 ± 3.6% and 89.3 ± 3.4% respectively (not significantly different). The prechallenge value of the control group was 103.5 ± 3.5%. The prechallenge values of all three groups on the exercise day were not significantly different from the 08.00 hours values on the control day.

Six children in group I, and 5 in group II developed an EOR after exercise (EIA+). There existed no significant differences in percentage fall from baseline value between the EIA+ children from the two groups. Mean values in percentage fall of these children from groups I and II were 28.5 ± 5.4% and 24.0 ± 1.8%, respectively.

None of the control children showed a fall in FEV_1 on exercise. Not a a single LOR after exercise was observed in any of the three groups.

Urinary catecholamine excretion

The adrenaline excretion values in the first two hours after HDM challenge showed no change compared with the values of the control day in either patient group, (Fig. 7.1) in spite of the severe degree of airflow obstruction in some patients. The decrease in noradrenaline excretion in the first two hours after HDM challenge as compared to values of the control day in group I (Fig. 7.1) was significant ($p<0.01$),

After exercise, in group I no increase and in group II a non-significant increase in catecholamine excretion in the first two hours after the provocation was observed, respectively (Fig. 7.1). A higher adrenaline and noradrenaline urinary excretion in group II was observed compared with the values of group I, although differences did not attain significance ($p>0.05$).

In the control group, a significant increase in adrenaline ($p<0.05$) as well as in noradrenaline ($p<0.01$) urinary excretion was observed. A significant difference

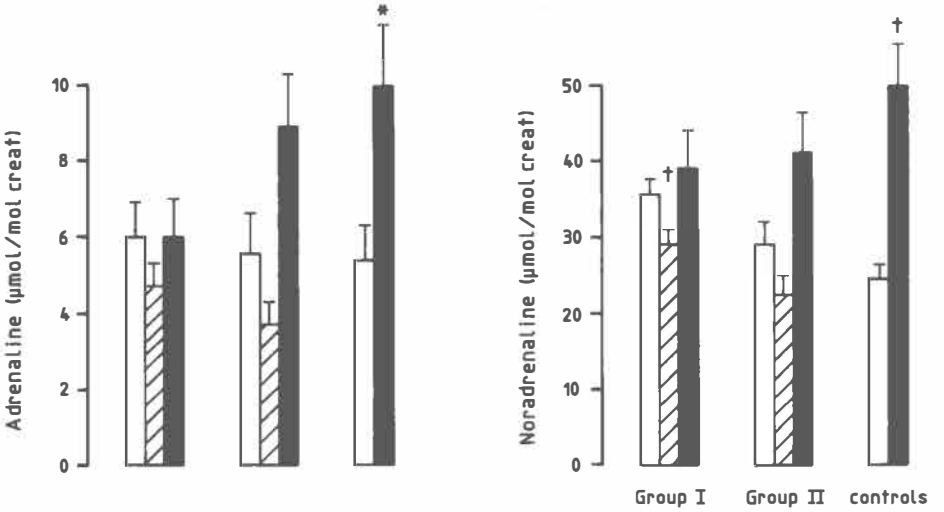


Figure 7.1: Adrenaline and noradrenaline excretion during the first two hours during and after the challenges. Open bars represent values of the control day, striped bars represent values of the house dust mite challenge day, and black bars represent values of the exercise day.

($p<0.05$) in urinary adrenaline excretion level existed between the control group and group I during the first two hours after exercise.

During the rest of both provocation days no differences in catecholamine excretion were found compared with the values on the control day of each group.

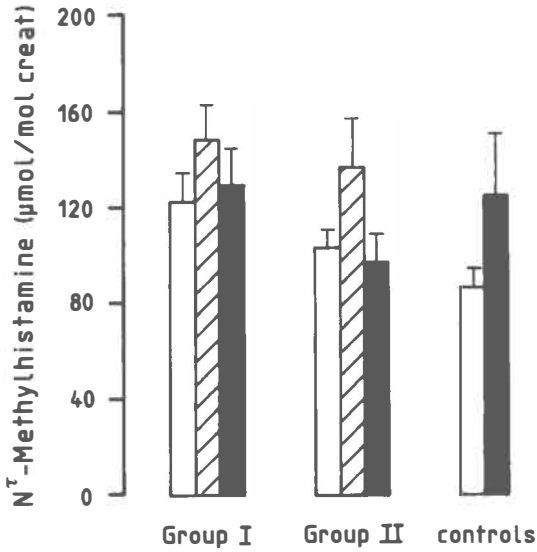


Figure 7.2: N$^{\tau}$-methylhistamine excretion during the first two hours during and after the challenges. Open bars represent values of the control day, striped bars represent values of the house dust mite challenge day, and black bars represent values of the

Urinary N^{τ}-methylhistamine excretion

Although a mean increase in N^{τ}-methylhistamine was observed during the first 2 sampling hours after HDM inhalation (Fig.7.2), this increase did just not reach significance in either group. During the LOR, no increases in N^{τ}-methylhistamine excretion were observed at all.

No differences in N^{τ}-methylhistamine excretion were observed after exercise compared with the control values of each group, during the complete 12 hours measurement period.

DISCUSSION

The results of this study show that the two groups of asthmatic patients had a comparable fall in FEV_1 during the EOR and LOR after HDM challenge. In 6 out of 9 children in group I (the group with increased airflow obstruction overnight), and in 5 out of 9 children in group II (the group without increased airflow obstruction overnight) exercise resulted in an EOR. Percentage fall in FEV_1 between the EIA+ patients from both groups were not significant. The observation that no differences existed between the two groups of asthmatic children in airflow obstructive patterns after either stimulus suggests that an increase in the amplitude of the circadian rhythm is more likely to be caused by other endogenous or exogenous factors.

We did not find any LORs after exercise, although almost all children had a LOR on HDM challenge. This is in contrast to the results of other investigators.[3,4] Moreover, in these studies a significant correlation between the degree of the EOR and the occurrence of a LOR existed. Our observations do not confirm this suggestion, as the fall in FEV_1 after exercise in our study (EIA+ children in group I: 28.5 ± 5.4%, and in group II: 24.0 ± 1.8%) is comparable with the results of these investigators (about 29% for reference 4 and 23.6 ± 4.0% for reference 5), while we did not observe any LOR. In addition, Lee et al.[3] and Bierman et al.[4] suggested that the LOR occurred in patients who do not appear to have a complete return to baseline FEV_1 values within three hours after completion of the test. An explanation for the differences in results may be that all children with an EOR in our study returned to their baseline FEV_1 values, or in most patients even to values above baseline, within two hours after exercise. It is not clear, however, why all our patients returned to baseline FEV_1 values, while the patients with a LOR of Lee et al. and Bierman et al. did not return to baseline values.

Rubenstein et al.[8], who observed a LOR only in 6 out of 53 patients with EIA, suggested that the large number of LORs after exercise observed by these investigators may be due to the fact that in these studies the control day preceded the exercise day, and that the observed LORs were, in fact, due to worsening of the pulmonary function after withdrawal of medication.

We observed a severe fall in FEV_1 values during the EOR after HDM and exercise challenge. Nevertheless we could not measure a significant increase in urinary catecholamine excretion after the two challenges in both patient groups. Despite the comparable strenuous exercise level in all three groups, only the healthy children showed a significant increase in urinary adrenaline excretion as well as urinary noradrenaline excretion after exercise. Our data suggest an impaired adrenal response during the EOR both after allergen inhalation and after exercise. Abnormally low plasma adrenaline levels have previously been observed in asthmatic patients who were admitted to hospital because of acute severe asthma.[9] The impaired response in our study appears not to stem from frequent use of beta-adrenergic drugs, since all but one of the asthmatic patients used these drugs only incidentally. Our findings confirm the data of Barnes et al.[10] They suggested that the circulating catecholamines have no direct role in exercise-induced airflow obstruction, but may play a permissive role via the mast cell. We could not, however, confirm this hypothesis, since we did not observe an increase in N^{τ}-methylhistamine excretion during and after exercise in either group of asthmatic children.

There was a striking difference in urinary catecholamine excretion level between the children from groups I and II after exercise, levels in the latter group being highest. This may stem from the fact that, although not significantly, FEV_1 and PC_{20} values in group I were obviously lower than the values of group II. Moreover, more children from group I used inhalation corticosteroids as compared to the children of group II. These clinical parameters indicate that the children of group I had more severe asthma than those in group II. Our observations that adrenaline excretion is significantly reduced in group I, but only slightly impaired in group II, suggests that the adrenergic response is gradually reduced on allergen and exercise challenge, depending upon the severity of asthma.

This may also explain the different results obtained by Chryssanthopoulos et al.[11], who observed that plasma levels of adrenaline at rest, and during and after exercise, were similar in asthmatics and in normal controls. The patients of Chryssanthopoulos et al. are, however, comparable to the children from our group II, but not to the children of group I. None of their patients had ever used corticosteroids, while most of our patients from group I used inhalation corticosteroids on a regular basis, in contrast to our patients in group II. In addition, the mean FEV_1%pred. value of their patients was 90%, which is comparable to the values of our group II.

After the first two hours, no differences in urinary catecholamine excretion existed during the rest of the day between the control and challenge days, despite the severe LOR after HDM challenge in most children. This again supports the idea that the beta adrenergic system is not sufficiently stimulated by an increase of airflow obstruction.

In both patient groups, urinary N^{τ}-methylhistamine excretion during the first two hours after HDM provocation showed a tendency to increase compared with the

control day. These findings point in the same direction as the observations of Keyzer et al.[12] and Löwhagen et al.[13] who showed a significant increase in urinary N^{τ}-methylhistamine excretion during the EOR by comparing differences between pre- and post challenge values on the control and the inhalation day. This indicates that the EOR after allergen challenge is accompanied by histamine release, suggesting direct involvement of mast cells. During the LOR upon HDM challenge, however, no changes in N^{τ}- methylhistamine excretion occurred in either patient groups compared with the control day. This corresponds with the data of others,[12,14,15] and confirm the general opinion that the mast cell is not directly involved in the LOR.

In 1981 Barnes et al.[16] observed that plasma histamine -levels after exercise were significantly higher in asthmatics than in normal controls, and suggested a discharge of mediators during exercise in asthmatics who developed exercise-induced asthma. Other investigators,[17,18] using more sensitive methods of histamine determination, did not find the EOR after exercise to be accompanied by increased histamine levels, indicating that in contrast to the increase in histamine after allergen challenge, airflow obstruction after exercise is achieved via another pathway. In accordance with these latter studies we did not observe any differences in urinary N^{τ}-methylhistamine excretion after exercise compared with the control day values in all three groups.

In conclusion, we observed no differences in airflow obstructive patterns after HDM challenge and exercise in the two groups of asthmatic children, indicating that both groups of children have the same ability to develop a LOR. Differences in environmental allergen exposure may rather influence the amplitude of the pulmonary function.

We could not confirm the observations of other investigators of LORs after exercise, in spite of the demonstrated ability of the patients to develop a LOR on allergen challenge.

An impaired adrenergic response in asthmatic children after both stimuli was established. The results from our two patient groups suggest that the degree of impairment is related to the severity of asthma. During and after the EOR on HDM challenge, a mean increase in N^{τ}-methylhistamine excretion existed. We did not observe an increase in N^{τ}-methylhistamine excretion during the LOR, indicating that histamine release is not of major importance in the development of airflow obstruction during the LOR.

REFERENCES

1. Newman-Taylor AJ, Davies RJ, Hendrick OJ, Pepys J. Recurrent nocturnal asthmatic reactions to bronchial provocation tests. Clin Allergy 1979;9:213-9.
2. Davies RJ, Green M, Schofield N. Recurrent nocturnal asthma after exposure to grain dust. Am Rev Resp Dis 1976;114:1011-9.
3. Lee TH, Nagakura T, Papageorgiou N, Likura Y, Kay AB. Exercise-induced late asthmatic reactions with neutrophil chemotactic activity. N Engl J Med 1983;308: 1502-5.
4. Bierman CW, Spiro SG, Petherham I. Characterization of the late response in exercise-induced asthma. J Allergy Clin Immunol 1984;74:701-6.
5. Cockroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in non-allergic bronchial reactivity. Clin Allergy 1977;7:503-13.
6. Godfrey S. Exercise testing in children. London: W.B.Saunders Co. Ltd, 1974.
7. Astrand PO, Rodahl K. Ciculation. In: Astrand PO, Rodahl K, eds. Textbook of work physiology: Physiological base of exercise. New York: McGraw-Hill Book Company, 1977;141-206.
8. Rubenstein I, Levison H, Slutsky AS, Hak H, Wells J, Zamel N, Rebuck AS. Immediate and delayed bronchoconstriction after exercise in patients with asthma. N Engl J Med 1987:8:482-5.
9. Ind PW, Causon RC, Brown MJ, Barnes PJ. Circulating catecholamine in acute asthma. Br Med J 1985;290:267-9.
10. Barnes PJ, Brown MJ, Silverman M, Dollery CT. Circulating catecholamines in exercise and hyperventilation induced asthma. Thorax 1981;36:435-40.
11. Chryssanthopoulos C, Barboriak SCD, Fink JN, Stekiel WJ Maksud MG. Adrenergic responses of asthmatic and normal subjects to submaximal and maximal work levels. J Allergy Clin Immunol 1978;61:17-22.
12. Keyzer JJ, Kauffman HF, De Monchy JGR, Keyzer-Uding JJ, De Vries K. Urinary N^{τ}-methylhistamine during early and late allergen-induced bronchial-obstructive reactions. J Allergy Clin Immunol 1984;74:240-5.
13. Löwhagen O, Granerus G, Wetterquist H. Studies on histamine metabolism in allergen-induced asthma. Allergy 1980;35:521-9.
14. Allen DH, Mathison DA, Wagner PD, Arroyave CM, Tan EM, Mediator release during allergen-induced bronchoconstriction in asthmatic subjects. Chest 1979;75:235-40.
15. Kauffman HF, Van der Heide S, De Monchy JGR, De Vries K. Plasma histamine concentrations and complement activation duringhouse dust mite-provoked bronchial-obstructive reactions. Clin Allergy 1983;13:219-28.
16. Barnes PJ, Brown MJ. Venous plasma histamine in exercise- and hyperventilation induced asthma in man. Clin Sci 1981;61:159-62.

17. Morgan DJR, Phillips MJ, Moodley I, Elliot EV, Davies RJ Histamine, neutrophil chemotactic factor and circulating basophil levels following exercise in asthmatic and control subjects. Clin Allergy 1982;12 (suppl):29-37.
18. Howarth PH, Pao GJK, Church MK, Holgate ST. Exercise and isocapnic hyperventilation-induced bronchoconstriction in asthma: Relevance of circulating basophils to measurements of plasma histamine. J Allergy clin Immunol 1984; 73:391-9.

SUMMARY AND CONCLUSIONS

A circadian variation in pulmonary function can be observed in normals with peak and trough values at 16.00 and 04.00 hours respectively.[1] Both in children and in adults with asthma the amplitude of the pulmonary function (i.e. the difference between the highest and lowest value measured over 24 hours expressed as percentage of the highest value) is increased and leads to nocturnal dyspnea.[2] The latter may awake asthmatics and influence everyday activities.

Recognition of increased nocturnal airflow obstruction can be difficult, and underlying mechanisms are only partly solved. Several studies in this field have been performed in adults, and since mechanisms may alter with increasing age, it is possible that in childhood other factors may contribute to nocturnal dyspnea than in adulthood. Evidence for this latter hypothesis is that certain drugs, that are of some help against nocturnal dyspnea in adults,[3] do not show the same results in children with comparable complaints.[4] In this thesis, studies were focused on the effects of bronchial hyperresponsiveness, autonomic regulation of bronchial smooth muscle tone, and of mediator release, on circadian changes in airway diameter.

The aims of the presented studies were:

1. to assess the effects of withdrawal of maintenance treatment on the circadian variation of lung function both at home and in hospital.
2. to investigate endogenous mechanisms that might contribute to nocturnal dyspnea, in order to attain a better understanding of the phenomenon.
3. to investigate whether an increased bronchial hyperresponsiveness at night was the consequence of increased airflowobstruction at night.
4 to investigate whether exogenous stimuli, particularly house dust mite (HDM) inhalation and exercise, influence the fall in pulmonary function overnight.
5. to investigate whether a PC_{10} or a PC_{15} histamine are as reproducible as a PC_{20} histamine in the assessment of the degree of bronchial hyperresponsiveness in asthmatic children.

In chapter 1, an introduction to chronobiology, and an overview of investigations concerning the subject of nocturnal dyspnea have been given. In this chapter the aims of the study are described in more detail.

In chapter 2 the selection procedure of the two groups of allergic asthmatic children, and of their normal controls has been reported. The selection of the two groups of asthmatic children was based on peak expiratory flow rate (PEFR) measurements at home, every four hours, on three consecutive days. In these two groups, a number of measurements were performed that took place in the hospital. The results of these investigations are reported in chapters 5, 6, and 7.

Group I, consisted of allergic asthmatic children with an amplitude in PEFR values

of 20% or more, and group II consisted of a group with an amplitude of less than 15%.

Other inclusion criteria for the two groups of asthmatic children were a forced expiratory volume in one second expressed as percentage of the predicted value (FEV_1%pred.) of at least 70% or more during the day, positive skin tests to at least HDM, and an increased bronchial responsiveness to histamine.
The children of group II and the normal controls were matched for age to the children of group I.

This chapter describes the complete study design (Fig. 1), the techniques of provocation tests, and laboratory determinations of adrenaline, noradrenaline, and N^{τ}-methylhistamine.

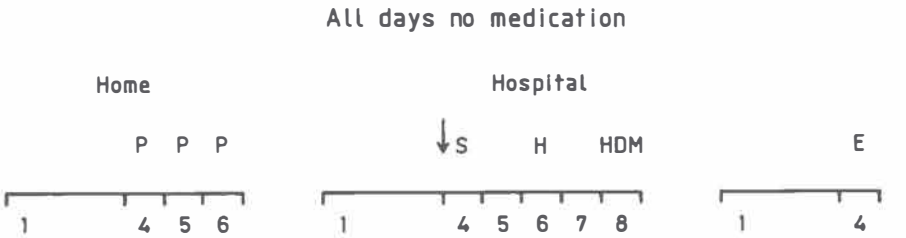


Figure 1: Study design of the two groups of patients. P=PEFR, S=spirometry, H=histamine inhalation provocation, HDM=house dust mite provocation, E=exercise test. The arrow indicates the first day of admission.

In chapter 3 we calculated whether PC_{10} and PC_{15} histamine are as reproducible as PC_{20} histamine in the assessment of the degree of bronchial responsiveness in asthmatic children. Therefore an inhalation provocation test with histamine was carried out on two consecutive days at 08.00 hours. We compared PC_{10}, PC_{15}, and PC_{20} values from twelve children on days 6 and 7 of the protocol and observed that all three indices of bronchial responsiveness were approximately equally reproducible. We concluded that a 10% fall in FEV_1 after histamine provocation is sufficient to estimate the degree of bronchial hyperresponsiveness in asthmatic children.

Clinical implications of this study are that once the diagnosis "CARA" (chronic a-specific respiratory affections) in children has been established, the follow up of bronchial hyperresponsiveness can be performed with a PC_{10} histamine. In children a PC_{10} instead of a PC_{20} may have the advantage of being less bothersome, and for the laboratory it may have the advantage of being less time-consuming. Another argument for using a PC_{10} instead of a PC_{20} in the assessment of the degree of bronchial hyperresponsiveness may be that in relatively healthy persons a PC_{20} can never be

reached due to the sigmoid shape of the dose-response curve with a maximum response plateau. To compare bronchial responsiveness in normals with asthmatic patients, the use of a PC_{10} may be of help.

This study does not provide an answer to the question whether a PC_{10} or PC_{15} histamine is sufficient to determine the cut-off between "asthma" and "normal" in a general population. Further investigations are necessary to investigate differences in "diagnostic value" of a PC_{10}, PC_{15}, and PC_{20} histamine.

In chapter 4 we discussed which factors could be of help to clinicians and patients in the recognition of nocturnal airflow obstruction. Therefore we investigated in well-controlled children with asthma whether it was possible to use daytime FEV_1 or PEFR measurements, or drugs needed to control the disease, to predict the decline in nocturnal PEFR values after withdrawal of all medication. We were interested whether pulmonary complaints, or decrease in PEFR values, were the first signs of deterioration in the disease state.

Three days before, and during the measurements all medication was withheld and the asthmatic patients performed PEFR measurements every 4 hours, for 3 days. Selection of the children for group I or II was based upon the amplitude in their PEFR values.

This study shows that it is not possible to predict from daytime FEV_1 and PEFR values alone, obtained during treatment, which asthmatic child would react with a nocturnal decline in PEFR values after withdrawal of medication.

The children with an amplitude of 20% or more showed an increase in amplitude of PEFR values when day 4, after withdrawal of maintenance medication, was compared with day 6. This increase was primarily caused by decreasing nocturnal PEFR values. We did not observe this phenomenon in group II.

We observed that children with a large amplitude, were more frequently dependent on inhaled corticosteroids compared with children with a small amplitude. There were no differences between the patients from group I and II in clinical history (all children werewell-controlled with their maintenance medication), nor in daytime pulmonary function values when on maintenance medication. The children in group I had already been considered as having more severe asthma. During the measurement days, we were surprised that only three out of thirty-six had to be considered as drop-outs. Although the children and parents were instructed to contact us and restart maintenance medication when the symptoms became troublesome, 47% of the participating children in the study showed a large decline in PEFR values overnight, but did not restart medication. This suggests that an increase in airflow obstruction overnight is underestimated by both parents and children.

History and daytime PEFR measurements shortly after withdrawal of medication provide insufficient information about the change in clinical disease state. Our advice is

to evaluate the effect of a reduced therapeutical regimen on the occurrence of nocturnal airflow obstruction by early morning (08.00 hours) PEFR measurements at home, after reduction of medication. Special attention should be paid to children using inhaled corticosteroids.

In chapters 5, 6, and 7 the results of studies that took place in the hospital are reported. All three groups consisted of 9 children.

In chapter 5 we investigated in the two groups of asthmatic children defined above, and their matched controls, whether endogenous factors, such as circadian changes in catecholamine production (measured as adrenaline and noradrenaline excretion in urine), circadian changes in vagal activity (indirectly measured as changes in heart rate and in sinus arrhythmia gap, the mean difference between maximum and minimum heart rate per minute), and circadian changes in histamine release (as measured by N^{τ}-methylhistamine), could contribute to the nocturnal fall in FEV_1 values.

We observed that the N^{τ}-methylhistamine excretion in group I was on a higher overall level compared with the values of both other groups, and that group II showed a higher level than the controls. In group I we found that N^{τ}-methylhistamine excretion increased during the night, in contrast to the excretion levels in both other groups. These data suggest that the increased airflow obstruction overnight is the result of an increased release of histamine and possibly other mediators overnight. Our data are in accordance with the preliminary results of Martin et al.[5], who, in adult patients with a nocturnal increase in airflow obstruction, observed an increase in white blood cell count, eosinophils, and neutrophils in bronchoalveolar lavage fluid obtained at 04.00 hours compared with the 16.00 hours values.

It is possible that this difference in N^{τ}-methylhistamine excretion between group I and II is exogenously determined. We hypothesize that environmental factors, and especially exposure to allergens during the day, induce inflammatory processes in the lung overnight. Another explanation may be that mean values of FEV_1 and PC_{20} histamine are lower in group I than in group II, although differences did not reach significant levels. The latter is possibly due to the small number of patients. More children in group I used inhaled corticosteroids than in group II. The above-mentioned findings suggest that the children from group I had more severe asthma than the children from group II. This difference is possibly determined by differences in exposure to stimuli at home.

We did not observe significant differences in variation of vagal activity between the three groups. This means that, in contrast to the observations in adults,[6] vagal activity in asthmatic children is not of importance to the increased airflow obstruction overnight. Our observation is supported by the finding that ipratropium bromide, an anticholinergic drug, does not prevent the nocturnal fall in FEV_1 values in asthmatic children,[4] while the drug is effective in adults.[3]

In addition, no major differences in catecholamine excretion were observed between the three groups. In all three groups daytime excretion levels were higher compared with nocturnal levels. This indicates that in allergic asthmatic children with nocturnal airflow obstruction catecholamine secretion is not stimulated by increased airflow obstruction and that the catecholamine levels are not sufficient to prevent the nocturnal fall in FEV_1 values.

In chapter 6 variations in bronchial hyperresponsiveness were discussed. The increase in bronchial hyperresponsiveness overnight coincides with the fall in FEV_1.[7] The aim of this study was to investigate whether an increase in bronchial hyperresponsiveness during the night was the consequence of a nightly decline in pulmonary function.

To answer this question, inhalation provocation tests with histamine were performed every 4 hours for 24 hours, starting at 08.00 hours. In both groups of asthmatic children we observed a nocturnal increase in the degree of bronchial hyperresponsiveness, despite the fact that the children from group II hardly showed a nocturnal fall in prechallenge FEV_1 values. This suggests that increased bronchial hyperresponsiveness is not by itself responsible for nocturnal dyspnea in asthmatic children. Thus the circadian variation in the degree of bronchial hyperresponsiveness seems to be an independent feature.

This study also shows that 4 hourly histamine inhalation challenges does not induce tachyphylaxis to histamine as has been reported in adult asthmatics.[8] Our 08.00 hours PC_{20} histamine values on days 6 and 7 of the study were comparable.

When in group I FEV_1 values on day 4, the first day of admission, were compared with prechallenge FEV_1 values of day 6, we observed a decrease in amplitude primarily due to increasing nocturnal FEV_1 values. This was in contrast to the observations at home where they showed a decrease in PEFR values. Again, no significant alterations appeared in the pulmonary function parameters of the children from group II.

The design of the study did not provide an answer to the question why asthmatic children with a large amplitude of pulmonary function parameters are more susceptible to environmental changes. It is possible that the group with the large amplitude is more exposed to airflow obstructive stimuli at home, and that these contacts during the day may lead to worsening of airflow obstruction at night.

In chapter 7 investigations have been reported concerning both groups of asthmatic children after challenge with HDM inhalation and exercise. Next to endogenous factors, exogenous stimuli may contribute to the increase in airflow obstruction overnight. We hypothesized that asthmatic children with increased nocturnal airflow obstruction overnight suffer more frequently from a late asthmatic reaction (LOR) on HDM and exercise challenge. We investigated whether differences between the two

groups of asthmatic children existed in airflow obstructive patterns after HDM inhalation and exercise. Moreover, we investigated whether these possible differences between the two groups were caused by differences in adrenaline and noradrenaline response, or by differences in N^{τ}-methylhistamine excretion.
The results of the investigations of both groups of asthmatic children were compared with values of their controls after exercise.

In the two asthmatic groups, no differences were observed in airflow obstructive pattern after both stimuli. All children showed an early, and almost all showed a late obstructive reaction (EOR and LOR, respectively) after HDM challenge. Five children from group I, and 6 from group II showed an EOR after exercise. No LORs were observed after exercise, in contrast to some observations described in the literature.[9,10]

In the two asthmatic groups, urinary catecholamine excretion was impaired after both challenges compared with the values of the healthy controls after exercise. Group II showed a higher response than group I. As discussed above, since group I may be considered as having more severe asthma than group II, it seems that the degree of impairment of catecholamine excretion is related to the severity of the disease. During the rest of the day, especially during the LOR after HDM challenge, no increase in catecholamine excretion was observed. This again, suggests that even severe airflow obstruction does not stimulate catecholamine secretion in asthmatic children.

We did not observe significant differences between the groups in N^{τ}-methylhistamine excretion during and after both stimuli. After HDM challenge, we observed during and after the EOR, a mean increase in urinary N^{τ}-methylhistamine excretion in both groups of asthmatic children. This observation points in the direction of other studies,[11,12] in which a significant increase was observed. We did not observe an increase in N^{τ}-methylhistamine excretion during the LOR, indicating that histamine release is not of major importance in the development of airflow obstruction during the LOR.

Final conclusions

A nocturnal fall in pulmonary function during the night is poorly recognized by asthmatic children and their parents.
Moreover, day-time PEFR and FEV_1 measurements obtained when on medication are not sufficient to predict the nocturnal decline in pulmonary function after withdrawal of medication. Follow-up of PEFR measurements after reduction of the therapeutical regimen at 08.00 hours may suggest the presence of increased nocturnal airflow obstruction.

The increase of histamine excretion overnight seems to be of importance to the development of the nocturnal fall in FEV_1 values in asthmatic children. The orthosympathetic system does not seem to be sufficient to prevent the fall in nocturnal

FEV1 values. The parasympathetic system is not of importance to noctural airflow obstruction in allergic asthmatic children. This is in contrast to adults with asthma or with chronic obstructive lung disease.

The circadian variation in bronchial hyperresponsiveness in children with and without increased airflow obstruction overnight is comparable, suggesting that the circadian variation in bronchial hyperresponsiveness is independent of the degree of airflow obstruction.

After HDM inhalation as well as after exercise we observed an impaired adrenergic response in both groups of asthmatic children, which seems to be related to the severity of the disease state and not to the degree of airflow obstruction due to the two stimuli.

Both groups of allergic asthmatic children showed comparable reactions to exogenous stimuli. Taken together with the observation that after withdrawal of medication at home the amplitude in pulmonary function increased, while in hospital the opposite happened, this suggests that nocturnal airflow obstruction may be determined by exogenous factors. It seems worthwile to continue investigations in this direction. Measurements of allergen concentrations in the normal surroundings of asthmatic children with and without nocturnal airflow obstruction may answer the question whether allergen exposure determines the increased amplitude in pulmonary function. In addition, studies in seasonal variations in the circadian amplitude of the pulmonary function may help to answer the question whether nocturnal airflow obstruction is exogenously determined.

REFERENCES

1. Gaultier C, Reinberg A, Girard F. Circadian rhythms in lung resistance and dynamic lung compliance of healthy children. Effects of two bronchodilators. Respiration Physiology 1977;31:169-82.
2. Hetzel MR, Clark TJH. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. Thorax 1980;35:732-8.
3. Cox ID, Hughes DTD, McDonnell KA. Ipratropium bromide in patients with nocturnal asthma. Postgr Med J 1984;60:562.
4. Sly PD, Landau LI, Olinski A. Failure of Ipratropium bromide to modify the diurnal variation of asthma in asthmatic children. Thorax 1987;42:357-60.
5. Martin RJ, Cicutto LC, Ballard RD, Szefler SJ. Airway inflammation and nocturnal asthma (Abstract). Am Rev Resp Dis 1988;137suppl:284.
6. Postma DS, Keyzer JJ, Koëter GH, Sluiter HJ, De Vries K. Influence of parasympathetic and sympathetic nervous system on nocturnal bronchial obstruction. Clin Sci 1985;69:251-8.
7. De Vries K, Goei JT, Booy-Noord H, Orie NGM. Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. Int Arch Allergy 1962;20:93-101.
8. Manning PJ, Jones GL, O'Byrne P. Tachyphylaxis to inhaled histamine in asthmatic subjects. J Appl Physiol 1987;63:1572-7.
9. Bierman CW, Spiro SG, Petherham I. Characterization of the late response in exercise-induced asthma. J Allergy Clin Immunol 1984;74:701-6.
10. Lee TH, Nagakura T, Papageorgiou N, Likura Y, Kay AB. Exercise-induced late asthmatic reactions with neutrophil chemotactic activity. N Engl J Med 1983;308: 1502-5.
11. Keyzer JJ, Kauffman HF, De Monchy JGR, Keyzer-Uding JJ, De Vries K. Urinary N^{τ}-methylhistamine during early and late allergen-induced bronchial obstructive reactions. J Allergy Clin Immunol 1984;74:240-5.
12. Löwhagen O, Granerus G, Wetterquist H. Studies on histamine metabolism in allergen-induced asthma. Allergy 1980;35:521-9.

SAMENVATTING

Normale personen hebben een circadiane (ongeveer 24 uur durende) variatie in hun longfunctie met piek en dal waarden om respectievelijk 16.00 en 04.00 uur.[1] Bij zowel kinderen als volwassenen met astma is de amplitude van de longfunctie (i.e. het verschil tussen de beste en slechtste waarde, gemeten over 24 uur en uitgedrukt als percentage van de beste waarde) toegenomen hetgeen aanleiding kan zijn tot nachtelijke kortademigheidsklachten.[2] Tot nu toe zijn factoren die zouden kunnen bijdragen aan de nachtelijke daling in de longfunctie slechts ten dele opgelost.

Zowel bij volwassenen als bij kinderen kan nachtelijke kortademigheid de nachtrust verstoren, en de dagelijkse activiteiten in negatieve zin beinvloeden.

De nachtelijke bronchusobstructie wordt zowel door de patient als door de dokter moeilijk herkend. Verschillende studies op dit terrein werden bij volwassenen verricht, en aangezien onderliggende mechanismen met het toenemen van de leeftijd kunnen veranderen, is het mogelijk dat op de kinderleeftijd andere factoren een rol spelen bij nachtelijke kortademigheid dan bij volwassenen. Een aanwijzing voor deze laatste hypothese is dat bepaalde geneesmiddelen goed helpen tegen nachtelijke kortademigheid bij volwassenen, terwijl ze dat niet doen bij kinderen.[3,4] De studies in dit proefschrift waren gericht op de effecten van bronchiale hyperreactiviteit, de autonome regulatie van de gladde spieren van de bronchiaalboom en het vrijkomen van mediatoren, op de circadiane verandering van de diameter van de luchtwegen.

Het doel van de verrichte studies was om:

1. de effecten op veranderingen in de 24-uurs longfunctie van het ontrekken van onderhoudsmedicatie thuis en in het ziekenhuis te onderzoeken.
2. endogene mechanismen te onderzoeken die aan nachtelijke kortademigheid kunnen bijdragen, om zodoende meer van dit fenomeen te kunnen begrijpen.
3. te onderzoeken of de nachtelijke toename van de bronchiale hyperreactiviteit het gevolg was van de toename in nachtelijke bronchusobstructie.
4. te onderzoeken of exogene prikkels, met name huisstofmijt inhalatie en inspanning, van invloed zijn op de nachtelijke daling van de longfunctie.
5. te onderzoeken of een PC_{10} of een PC_{15} histamine even reproduceerbaar zijn als een PC_{20} histamine, bij het bepalen van de mate van bronchiale hyperreactiviteit bij kinderen met astma.

In hoofdstuk 1 wordt een inleiding en een overzicht over chronobiologie in relatie tot nachtelijke kortademigheid gegeven. In dit hoofdstuk wordt ook het doel van de studies beschreven.

In hoofdstuk 2 wordt de selectie procedure van de twee groepen allergische kinderen met astma en hun gezonde controles beschreven. Twee groepen kinderen met astma werden geselecteerd op basis van piek stroom (PEFR) metingen die gedurende

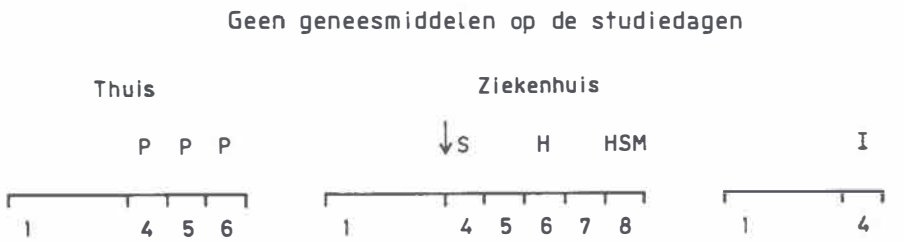
drie achtereenvolgende dagen thuis, elke 4 uur, verricht werden. Bij deze twee groepen, beide bestaande uit 9 kinderen, werd in het ziekenhuis het onderzoek uitgevoerd. De resultaten zijn in de hoofdstukken 5, 6 en 7 beschreven.

Groep I bestond uit een groep allergische kinderen met astma, met een amplitude in PEFR waarden van 20% of meer en groep II bestond eveneens uit een groep allergische kinderen met astma, maar met een amplitude van minder dan 15%.

Andere insluitingscriteria voor de twee groepen astmatische kinderen waren een overdag gemeten geforceerd een-seconde volume (FEV_1) uitgedrukt als percentage van de voorspelde waarde van tenminste 70%, allergie voor huisstofmijten, en een toegenomen prikkelbaarheid voor histamine.

De kinderen van groep II en de controles waren gematched met groep I op basis van de leeftijd.

In dit hoofdstuk worden het volledige studieprotocol (Fig. 1), de provocatietechnieken en de laboratoriumbepalingen van adrenaline, noradrenaline en N^{τ}-methylhistamine beschreven.



Figuur 1: studie protocol van de twee patienten groepen. P=PEFR, H=histamine inhalatie provocatie, HSM=huisstofmijt provocatie, I=inspannings test. De pijl geeft de eerste opname dag aan.

In hoofdstuk 3 berekenden we of een PC_{10} en een PC_{15} histamine even reproduceerbaar zijn als de PC_{20} bij het bepalen van de mate van bronchiale prikkelbaarheid bij kinderen met astma. Er werden op twee opeenvolgende dagen om 8.00 uur histamine-inhalatie-provocatie tests verricht. We vergeleken PC_{10}, PC_{15} en PC_{20} waarden van 12 kinderen op dag 6 en 7 van het protocol, en zagen dat deze drie parameters ongeveer even reproduceerbaar zijn. We concludeerden dat een daling van 10% in FEV_1 waarden na histamine provocatie voldoende is om de mate van bronchiale hyperreactiviteit bij

kinderen met astma te bepalen.

De klinische implicatie van deze studie is dat wanneer eenmaal de diagnose CARA (chronische aspecifieke respiratoire aandoeningen) bij kinderen is gesteld, het vervolgen van de mate van bronchiale hyperreactiviteit kan gebeuren middels een PC_{10} van histamine. Bij sommige kinderen zou dit als voordeel kunnen hebben dat de provocatie minder hinderlijk is en voor het longfunctie-laboratorium is het een voordeel dat de provocatie-procedure iets minder lang duurt. Een ander argument voor het gebruik van een PC_{10} in plaats van een PC_{20} bij het bepalen van de mate van bronchiale hyperreactiviteit is dat bij relatief gezonde personen een PC_{20} nooit gehaald wordt door de sigmoide vorm van de dosis-respons curve met een maximaal respons plateau. Om de mate van bronchiale reactiviteit van gezonden en patienten met astma te kunnen vergelijken, zou het gebruik van een PC_{10} uitkomst bieden.

Dit onderzoek geeft geen antwoord op de vraag of een PC_{10} of een PC_{15} voldoende is om de scheiding tussen personen met en zonder astma te maken. Verder onderzoek is noodzakelijk om verschillen in de diagnostische waarde van een PC_{10}, een PC_{15} en een PC_{20} van histamine te bepalen.

In hoofdstuk 4 wordt besproken welke parameters bij het herkennen van toegenomen nachtelijke bronchusobstructie behulpzaam kunnen zijn. Om deze reden werd bij goed medicamenteus ingestelde kinderen met astma onderzocht of het mogelijk was met behulp van overdag gemeten FEV_1 en PEFR waarden, of op geleide van de geneesmiddelen die nodig zijn om de ziekte onder controle te houden, de verslechtering te voorspellen van nachtelijke PEFR waarden nadat alle geneesmiddelen gestopt werden. We wilden weten of benauwdheidsklachten, of een vermindering van nachtelijke PEFR waarden, de eerste tekenen van verslechtering zouden zijn.

Drie dagen voor en tijdens de metingen die thuis plaats vonden, werden alle geneesmiddelen gestopt. Door de kinderen werden elke 4 uur, gedurende drie opeenvolgende dagen, PEFR metingen verricht. Op geleide van de amplitude van hun PEFR waarden werden de kinderen in groep I of II ingedeeld.

Dit onderzoek laat zien dat het niet mogelijk is om op basis van overdag bepaalde FEV_1 of PEFR waarden, gemeten terwijl de kinderen nog op hun onderhoudsmedicatie ingesteld waren, te voorspellen welk kind zal reageren met een nachtelijke daling van de PEFR waarden na het stoppen van die medicatie.

De kinderen met een amplitude van meer dan 20%, lieten na het staken van de medicatie een toename van de amplitude in PEFR waarden zien wanneer dag 4 met dag 6 vergeleken werd. Deze toegenomen amplitude werd voornamelijk veroorzaakt doordat de nachtelijke waarden afnamen. We zagen dit niet bij de kinderen uit groep II gebeuren.

Meer patienten met een grote amplitude dan die met een kleine amplitude bleken inhalatie corticosteroiden te gebruiken. Dit geeft aan dat, hoewel er geen anamnestische

verschillen waren tussen de patienten van groep I en II (alle kinderen waren goed ingesteld op hun onderhoudsmedicatie) en er geen verschillen waren in overdag gemeten longfunctiewaarden voordat de medicatie gestopt werd, deze kinderen in het verleden, vermoedelijk op klinische gronden, als ernstiger astma patienten beschouwd werden. Het verrastte ons dat gedurende de metingen slechts drie van de zesendertig kinderen uitvielen. Hoewel de patienten en hun ouders geinstrueerd waren om contact met ons op te nemen, en de medicatie weer te hervatten wanneer er benauwdheidsklachten optraden, vertoonde 47% van de kinderen die meededen, een behoorlijke daling van hun PEFR waarden 's nachts terwijl ze hun medicatie toch niet hervatten. Dit houdt in dat de toename in bronchusobstructie 's nachts onderschat wordt door zowel ouders als kinderen.

Anamnese en overdag gemeten PEFR waarden kort nadat de medicatie gestopt wordt, geven onvoldoende informatie over de verandering van het ziektebeeld. We adviseren om het effect van een verminderd therapeutisch regime op het ontwikkelen van nachtelijke bronchusobstructie te evalueren door thuis, s'morgens vroeg (08.00 uur), de PEFR waarden te vervolgen.
Hierbij dient speciaal gelet te worden op kinderen die ingesteld zijn op inhalatie corticosteroiden.

In de hoofdstukken 5, 6 en 7 wordt over de resultaten van de studies die in het ziekenhuis plaatsvonden gerapporteerd. Alle drie groepen bevatten 9 kinderen.

In hoofdstuk 5 onderzochten we bij de twee hierboven gedefinieerde groepen kinderen met astma en bij hun controle groep, in hoeverre endogene factoren zoals circadiane veranderingen in catecholamine productie, gemeten als adrenaline en noradrenaline uitscheiding in de urine, circadiane veranderingen in vagusactiviteit, indirect gemeten als verandering in de hartfrequentie en "sinus arrhythmia gap" (het gemiddelde verschil tussen maximum en minimum hart frequentie per minuut), en circadiane veranderingen in het vrijkomen van histamine, gemeten als de uitscheiding van N^{τ}-methylhistamine in de urine, kunnen bijdragen tot de nachtelijke daling van FEV_1 waarden.

We zagen dat de N^{τ}-methylhistamine-uitscheiding bij groep I over de hele linie groter was dan in beide andere groepen, en bij groep II was deze weer groter dan bij de controles. We vonden bij groep I dat de N^{τ}-methylhistamine-uitscheiding 's nachts toenam, in tegenstelling tot bij beide andere groepen. Deze resultaten suggereren dat de nachtelijke toegenomen bronchusobstructie het gevolg is van het 's nachts in toegenomen mate vrijkomen van histamine en mogelijk andere mediatoren. Onze bevindingen stemmen overeen met de onderzoeksresultaten van Martin et al.[5] die bij volwassenen met een nachtelijke daling in FEV_1 waarden, in de broncho-alveolaire lavagevloeistof van 04.00 uur een toename zagen in aantal leucocyten, eosinofiele en

neutrofiele granulocyten, wanneer de aantallen vergeleken werden met die van 16.00 uur.

Het verschil in N^{τ}-methylhistamine-uitscheiding zou exogeen bepaald kunnen zijn. We veronderstellen dat omgevingsfactoren overdag, en allergeen inhalatie in het bijzonder, 's nachts ontstekingsprocessen in de luchtwegen induceren. Een andere verklaring voor het verschil in N^{τ}-methylhistamine uitscheiding zou kunnen zijn dat FEV_1 en PC_{20} histamine waarden van groep I lager zijn dan van groep II, hoewel het verschil niet significant is. Mogelijk dat dit laatste het gevolg is van het kleine aantal patienten in de groepen. Bovendien gebruiken meer kinderen van groep I inhalatie corticosteroiden. Deze gegevens suggereren dat de kinderen uit groep I ernstiger astma hebben dan de kinderen uit groep II. Het is mogelijk dat dit verschil in ernst van de ziekte bepaald wordt door verschillen in expositie aan prikkelende factoren thuis.

We zagen geen verschillen in variatie in vagusactiviteit tussen de drie groepen. Dit betekent dat vagusactiviteit, in tegenstelling tot bij volwassenen,[6] bij kinderen met asthma geen rol van betekenis speelt bij de toegenomen nachtelijke bronchusobstructie. Onze bevindingen worden ondersteund door de resultaten van andere onderzoekers, dat ipratropiumbromide, een anticholinergisch werkend geneesmiddel, de daling in FEV_1 waarden 's nachts bij kinderen met astma niet voorkomt,[4] terwijl dit wel het geval is bij volwassenen.[3]

Ook werden geen verschillen van betekenis gevonden in catecholamine-uitscheiding tussen de drie groepen. Bij alle groepen zagen we dat de uitscheiding overdag hoger was dan 's nachts. Dit geeft aan dat bij allergische kinderen met astma de catecholaminesecretie niet gestimuleerd wordt door toename van de mate van bronchusobstructie en dat de catecholamine- spiegels onvoldoende zijn om de nachtelijke daling in FEV_1 waarden te voorkomen.

In hoofdstuk 6 worden veranderingen in bronchiale hyperreactiviteit besproken. De toename in bronchiale hyperreactivieit 's nachts vindt op hetzelfde tijdstip plaats als de daling in FEV_1 waarden.[7] Het doel van deze studie was om te onderzoeken of de nachtelijke toename in bronchiale hyperreactiviteit het gevolg is van de nachtelijke daling van de longfunctie.

Om deze vraag te beantwoorden werden elke 4 uur, gedurende 24 uur histamine-inhalatie provocatie-tests verricht. De provocaties begonnen om 08.00 uur. We zagen in beide groepen kinderen met astma, een nachtelijke toename in de mate van bronchiale hyperreactiviteit, ondanks dat de kinderen uit groep II nauwelijks enige daling in FEV_1waarden voorafgaande aan de provocatie tests lieten zien. Deze bevinding suggereert dat toename van de bronchiale hyperreactiviteit op zich niet verantwoordelijk is voor nachtelijke kortademigheidsklachten bij kinderen met astma, en het lijkt erop dat de circadiane variatie in de mate van bronchiale hyperreactiviteit een fenomeen is, dat onafhankelijk is van de verandering in longfunctie.

Deze studie laat tevens zien dat wanneer histamine-inhalatie provocatie-tests bij kinderen met astma elke 4 uur herhaald worden er geen tachyphylaxie geinduceerd wordt, zoals dat wel gemeld wordt bij volwassenen met astma.[8] De 08.00 uurs waarden van de PC_{20} histamine op studiedag 6 en 7 waren vergelijkbaar.

In groep I zagen we dat wanneer dag 4, de eerste opname dag, vergeleken werd met dag 6, er een afname in de amplitude van FEV_1 waarden was. Dit was in tegenstelling tot de bevindingen thuis, waar juist een toename in de amplitude van PEFR waarden gevonden werd. Er traden wederom geen significante veranderingen op in de longfunctieparameters van groep II.

Waarom kinderen met astma met een grote amplitude van hun longfunctieparameters gevoeliger zijn voor verandering van omgeving is niet duidelijk. Mogelijk dat deze groep thuis meer aan bronchusobstructieve prikkels blootgesteld is, en dat deze contacten overdag aanleiding zijn voor nachtelijke bronchusobstructie.

Behalve endogene factoren, kunnen ook exogene factoren bijdragen tot de nachtelijke toename in bronchusobstructie.
In hoofdstuk 7 wordt onderzoek besproken waarbij beide groepen kinderen met astma met huisstofmijt inhalatie en inspanning geprovoceerd werden. Verondersteld werd dat kinderen met astma die 's nachts ernstiger bronchusobstructief waren, vaker late obstructieve reacties (LOR) doormaken. We onderzochten of er verschillen bestonden in het bronchusobstructieve reactiepatroon nadat beide groepen kinderen met astma geprovoceerd werden met huisstofmijt inhalatie en inspanning. Bovendien werd onderzocht of de mogelijke verschillen tussen de groepen kinderen met astma veroorzaakt werden door verschillen in adrenaline en noradrenaline respons, of in verschillen in N^{τ}-methylhistamine uitscheiding.

De resultaten van het onderzoek bij beide groepen kinderen met astma werden vergeleken met de waarden van de controle groep na inspanning.

Er werden in beide groepen kinderen met astma geen verschillen in bronchusobstructief reactiepatroon gezien na het toedienen van beide prikkels. Na huisstofmijtinhalatie lieten alle kinderen een vroege, en bijna alle kinderen een late obstructieve reactie (EOR en LOR) zien. Vijf kinderen uit groep I en 6 uit groep II vertoonden een EOR na inspanning. In tegenstelling tot andere bevindingen in de literatuur werd geen LOR na inspanning gevonden.[9,10]

De catecholamine uitscheiding in de urine was na beide provocaties minder in beide groepen kinderen met astma, in vergelijking met de waarden van de controles na inspanning. Groep II had een grotere respons dan groep I. Aangezien groep I, zoals al eerder besproken werd, beschouwd kan worden als de groep met ernstiger astma, lijkt het erop dat de mate van beperking van catecholamine-uitscheiding gerelateerd is aan de ernst van de ziekte.

Gedurende de rest van de dag, en in het bijzonder gedurende de LOR na huisstof-

mijt-provocatie, werd geen toename van catecholamine uitscheiding gezien. Deze bevinding suggereert opnieuw dat bronchusobstructie, zelfs als deze ernstig is, de catecholaminesecretie niet stimuleert.

Er werden geen significante verschillen in N^{τ}-methylhistamine uitscheiding gezien tussen de drie groepen gedurende en nadat de prikkels waren toegediend. Tijdens de EOR na huisstofmijt provocatie werd bij beide groepen kinderen met astma een gemiddelde, niet significante stijging gezien. Deze bevinding wijst in de richting van onderzoeks resultaten van anderen,[11,12] waarbij wel een significante stijging gezien werd. Er werd geen toename van N^{τ}-methylhistamine uitscheiding gezien tijdens de LOR. Deze bevindingen suggereren dat de EOR, in tegenstelling tot de LOR, gepaard gaan met het vrijkomen van histamine.

Slot conclusies

Wij concluderen dat de nachtelijke daling in de longfunctie door patienten en hun ouders onvoldoende herkend wordt, en dat overdag gemeten PEFR en FEV_1 waarden onvoldoende zijn om de nachtelijke verslechtering in de longfunctie bij kinderen met astma te voorspellen wanneer de medicatie gestopt wordt. Het vervolgen van PEFR metingen om 08.00 uur, nadat een therapeutisch regime veranderd is, kan het optreden van nachtelijke bronchusobstructie herkenbaar maken.

De toename in histamine-uitscheiding 's nachts lijkt van belang te zijn voor het ontstaan van de nachtelijke daling in FEV_1 waarden. De orthosympatische activiteit is onvoldoende om de nachtelijke daling in FEV_1 waarden te voorkomen. Vagus activiteit is, in tegenstelling tot bij volwassenen met astma, niet van belang voor de nachtelijke toename in bronchusobstructie.

De variatie over 24 uur in bronchiale hyperreactiviteit bij kinderen met en zonder toegenomen bronchusobstructie 's nachts is vergelijkbaar, hetgeen suggereert dat de circadiane variatie in de mate van bronchiale hyperreactiviteit onafhankelijk is van de mate van bronchusobstructie.

We zagen dat na huisstofmijt-inhalatie en na inspanning er een verzwakte adrenerge respons was bij beide groepen kinderen met astma, die gerelateerd lijkt te zijn aan de ernst van de ziekte en niet aan de mate van bronchusobstructie tengevolge van beide prikkels.

Beide groepen allergische kinderen met astma vertoonden vergelijkbare reacties op exogene prikkels. Dit, tesamen met de bevinding dat na het stoppen van de medicatie thuis de amplitude van de longfunctie toenam, terwijl in het ziekenhuis het omgekeerde gebeurde, suggereert dat nachtelijke bronchusobstructie bepaald wordt door exogene factoren. Het is de moeite waard om toekomstig onderzoek hierop te richten. Metingen van allergeenconcentraties in de leefomgeving van kinderen met en zonder toegenomen bronchusobstructie 's nachts zouden de vraag kunnen beantwoorden of allergeen

expositie inderdaad de toename in amplitude van de longfunctie bepaalt. Ook seizoensvariaties in de circadiane amplitude van de longfunctie kunnen meer inzicht verschaffen over het exogeen bepaald zijn van de nachtelijke daling in de longfunctie.

LITERATUUR

1. Gaultier C, Reinberg A, Girard F. Circadian rhythms in lung resistance and dynamic lung compliance of healthy children. Effects of two bronchodilators. Respiration Physiology 1977;31:169-82.
2. Hetzel MR, Clark TJH. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. Thorax 1980;35:732-8.
3. Cox ID, Hughes DTD, McDonnell KA. Ipratropium bromide in patients with nocturnal asthma. Postgr Med J 1984;60:562.
4. Sly PD, Landau LI, Olinski A. Failure of ipratropium bromide to modify the diurnal variation of asthma in asthmatic children. Thorax 1987;42:357-60.
5. Martin RJ, Cicutto LC, Ballard RD, Szefler SJ. Airway inflammation and nocturnal asthma (Abstract). Am Rev Resp Dis 1988;137suppl:284.
6. Postma DS, Keyzer JJ, Koëter GH, Sluiter HJ, De Vries K. Influence of parasympathetic and sympathetic nervous system on nocturnal bronchial obstruction. Clin Sci 1985;69:251-8.
7. De Vries K, Goei JT, Booy-Noord H, Orie NGM. Changes during 24 hours in the lung function and histamine hyperreactivity of the bronchial tree in asthmatic and bronchitic patients. Int Arch Allergy 1962;20:93-101.
8. Manning PJ, Jones GL, O'Byrne P. Tachyphylaxis to inhaled histamine in asthmatic subjects. J Appl Physiol 1987;63:1572-7.
9. Bierman CW, Spiro SG, Petherham I. Characterization of the late response in exercise-induced asthma. J Allergy Clin Immunol 1984;74:701-6.
10. Lee TH, Nagakura T, Papageorgiou N, Likura Y, Kay AB. Exercise-induced late asthmatic reactions with neutrophil chemotactic activity. N Engl J Med 1983;308: 1502-5.
11. Keyzer JJ, Kauffman HF, De Monchy JGR, Keyzer-Uding JJ, De Vries K. Urinary N^{τ}-methylhistamine during early and late allergen-induced bronchial obstructive reactions. J Allergy Clin Immunol 1984;74:240-5.
12. Löwhagen O, Granerus G, Wetterquist H. Studies on histamine metabolism in allergen-induced asthma. Allergy 1980;35:521-9.